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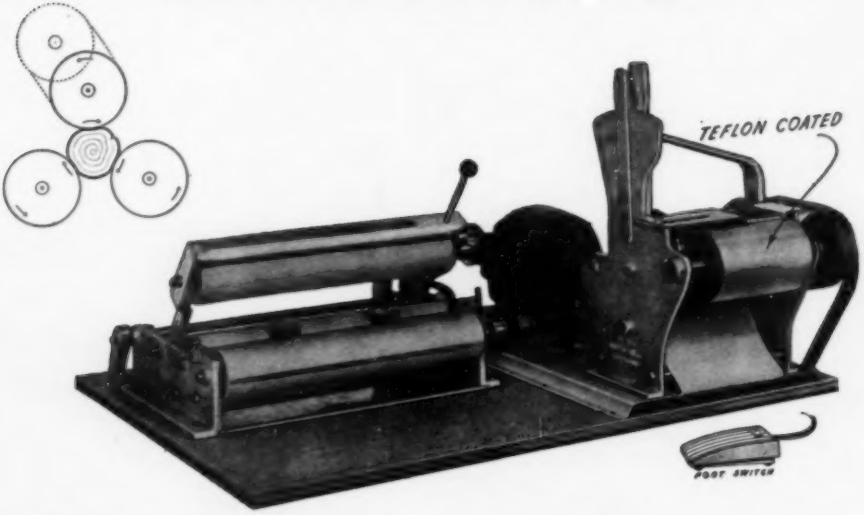

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INSIDE SCIENCE

The Vital Story of Vitamin B_1

(Thiamine)

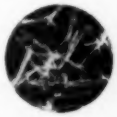
by Science Writer

History. The discovery of vitamin B_1 resulted from research into the cause of beriberi. Almost 50 years passed between Eijkman's discovery of the relationship of the disease to diet and the famous work of Jansen and Donath who first isolated the crystalline vitamin from rice bran.

Within ten years of that first isolation the vitamin's chemical structure was determined and it was successfully synthesized.

Eijkman's work resulted in the development of a theory that beriberi was caused by a lack of some factor in the diet and not by a toxin or infectious agent. This idea was not readily accepted until the growth of dietary knowledge proved it correct.

Isolation and Synthesis. In 1926 Profs. Jansen and Donath accomplished the isolation of crystalline vitamin B_1 from rice bran. In 1931 Windaus and coworkers successfully isolated pure vitamin B_1 and established its empirical formula. In 1936 R. R. Williams, and independently R. Grewe, explained the vitamin's chemical structure. That year, R. R. Williams and J. K. Cline accomplished the synthesis of thiamine which is in wide use today. Andersag and Westphal also synthesized the vitamin in 1936. Another synthesis was described by Bergel and Todd in 1937.



Photomicrograph of B_1 crystals

Chemical and Physical Properties. Thiamine hydrochloride is white, water soluble, with a nut-like, salty taste and yeast-like odor. Its empirical formula is: $C_{12}H_{17}ClN_4OS \cdot HCl$. Thiamine produced by synthesis is identical chemically and in biological activity with that obtained in pure form from nature.

Deficiencies. A deficiency of thiamine is characterized by these symptoms: depression, irritability, fearfulness, lack of initiative and interest, loss of appetite. Symptoms vary since in usual practice deficiencies of other water-soluble vitamins occur. Medical treatment is simple: a sufficient amount of thiamine is administered to relieve symptoms quickly and the physician provides for a continuing adequate intake.

Beriberi victim

A severe deficiency of thiamine leads to beriberi, a serious and sometimes fatal disease. While beriberi is almost a medical curiosity in the United States, it is common in countries in which polished white rice is a staple of the diet.

Human Nutrition Requirements. Thiamine is one of the nutritive elements the human body needs daily and does not store in quantity. The minimum daily requirements established by the U. S. Food and Drug Administration for the prevention of symptoms of thiamine deficiency disease are:

Adults ....1.00 mg.	Children (1-5 incl.) . .0.50 mg.
Infants ....0.25 mg.	Children (6-11 incl.) .0.75 mg.

The Food and Nutrition Board of the National Research Council recommends the following dietary intake of thiamine for healthy persons in the U. S. A.

Recommended Daily Intake in Milligrams

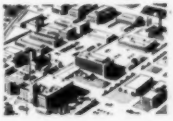
Age	*Men*	*Women*
25	1.6	1.2
45	1.5	1.1
65	1.3	1.0
Pregnant (3rd trimester)		1.5
Lactating		1.5

The Council recommendations for infants and children vary below and above these figures, based on age and sex. Various illnesses and stress situations can exhaust vital reserves of thiamine. So, for the physician, vitamin B_1 is prepared in various dosage forms and potencies for therapeutic and prophylactic use.

How do human beings receive thiamine? It is widely distributed in foods of animal and vegetable origin, particularly cereal grains and dry legumes. Because of public demand for refined products which millers must meet for obvious economic reasons, a loss of thiamine and other factors occurs during processing. The thiamine loss is overcome through the use of *enrichment* in cereal grain products for which Federal Standards exist, or in other foods such as breakfast cereals, by *fortification* or *restoration*. When enriching, fortifying or restoring, the food processor adds the necessary amount of pure thiamine (and other vitamins and minerals) to the food so that the finished product meets Federal, state and territorial requirements or contributes to the consumer an amount of the vitamin which dietary experts believe significantly useful.

Thiamine is extensively used for the enrichment of cereal grain foods such as white flour, white bread and rolls, macaroni products, farina, corn grits and meal, milled white rice. The story of these uses is delightfully told in a separate brochure which is available on request for reference or educational purposes.

Production. Huge production facilities at the Hoffmann-La Roche plant in Nutley, New Jersey, deliver highest quality thiamine *by the tons*. Roche manufactures thiamine hydrochloride and thiamine mononitrate. These fine products, which equal or exceed U.S.P. specifications, are ideal for use by pharmaceutical makers and food processors. Years of experience in research and manufacture have made Roche the leader in vitamins.



This article is published in the interests of pharmaceutical manufacturers, and of food processors who make their good foods better with essential, health-giving vitamin B_1. Reprints of this and others in the series are available on request. Write the Vitamin Division, Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul Street, West; Montreal, Quebec.

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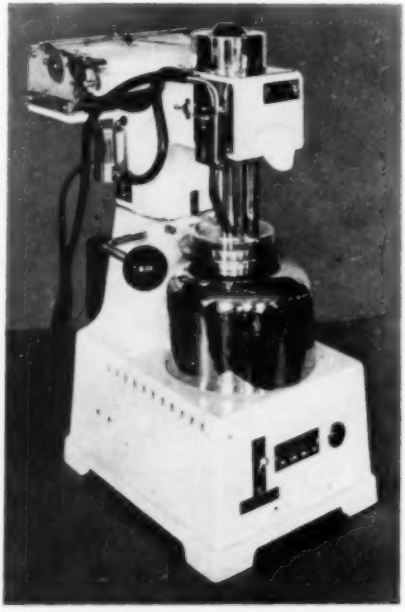

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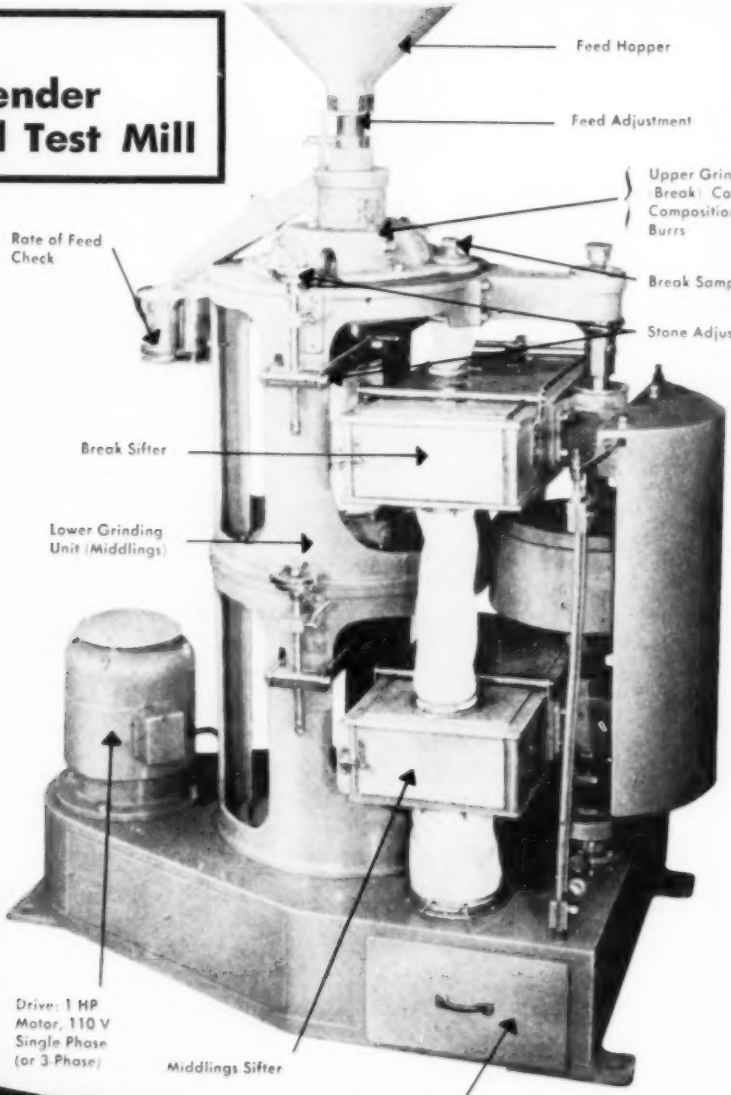

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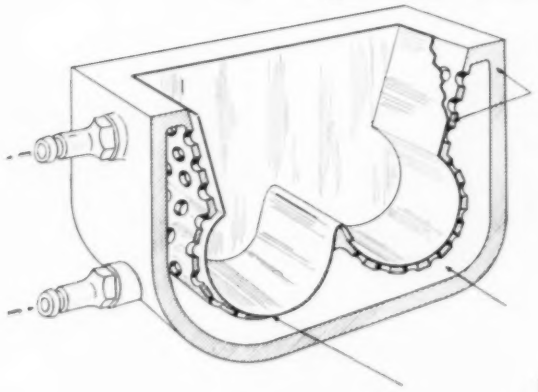

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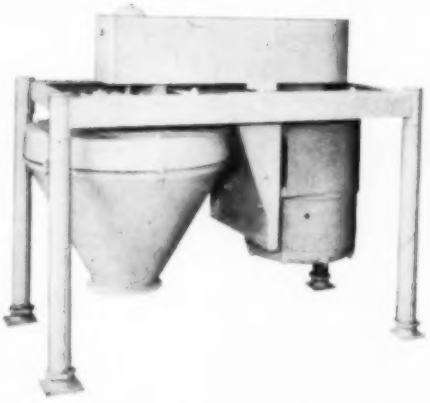

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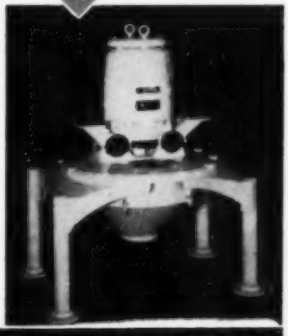

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CEREAL CHEMISTRY

VOL. 33 SEPTEMBER, 1956 NO. 5

CORRELATION OF FARINOGRAPH, MIXOGRAPH, SEDIMENTATION, AND BAKING DATA FOR HARD RED WINTER WHEAT FLOUR SAMPLES VARYING WIDELY IN QUALITY[1]

BYRON S. MILLER,[2] BETTE HAYS,[2] AND JOHN A. JOHNSON[2]

ABSTRACT

Quality tests were performed on flour from five hard red winter wheat varieties grown at each of twelve locations in Kansas during 1953–54 and on flour from eight varieties grown at seventeen locations in Kansas in 1954–55. These samples represent an extreme range in quality characteristics. Although these samples may have somewhat abnormal quality characteristics due to the environmental conditions during ripening, they provide useful material for study of the relationships among various physical dough measurements.

Correlation analyses through analyses of covariance were made of the quality data. Experimental baking mixing time was more highly correlated with mixograph mixing time than with farinograph mixing time. Variety significantly affected the correlation between experimental baking mixing time and mixograph mixing time, but the effect of location on this relationship depended on the season. The correlation of experimental baking mixing time and sedimentation value was of low order, as was also the correlation between farinograph and mixograph mixing time.

There was no significant relationship between the mixing tolerance index determined from the farinograms and the weakening angle determined from the mixograms. In view of the established relationship of the weakening angle to mixing sensitivity of the flour, the weakening angle may be a better measure of the sensitivity of a flour to mixing than the mixing tolerance index.

The only relationships sufficiently correlated for predictions were baking and mixograph mixing time, flour protein and sedimentation value, and valorimeter value and sedimentation value. These relationships may be valuable to those who need rapid and simple tests to measure flour quality.

The demand by bakers for flour with specified physical dough properties has forced cereal chemists to rely mainly upon data from either the farinograph or mixograph. The farinograph is a precision instrument and has been at least partly standardized. The mixograph is not a precision instrument and no attempt has been made to stand-

[1] Manuscript received March 12, 1956. Cooperative investigations between the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and the Department of Flour and Feed Milling Industries, Kansas State College. Contribution No. 267, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan.

[2] Chemist, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and Technician and Professor, Department of Flour and Feed Milling Industries, Kansas State College, Manhattan, respectively.

ardize it. The farinograph has been used more extensively than the mixograph. Both instruments have been used to indicate how a flour should be treated in the bakery for best baking results, although definite information on how well the instruments can predict optimum treatment in the bakery is not known with certainty.

Numerous studies have established that the farinograph provides an accurate estimate of flour absorption (14, 18, 19). Absorption, as determined by the farinograph, has been found to be highly correlated with the strength and protein content of the flour (4, 13, 15). The farinograph also has been suggested as a means of determining mixing requirement and sensitivity to mixing (18). It has been widely accepted for this use by the cereal industry (16).

The valorimeter value obtained from farinograms has been used as an index of flour strength and found to be correlated positively with protein content (8). Another farinogram characteristic, the mixing tolerance index (2, 17), is widely used by millers and bakers as a measure of the tolerance of a flour to overmixing. No data are available to indicate its relationship to performance of flours in the bakery.

Excellent correlations between flour protein and loaf volume have been reported (5, 10, 11). These data were obtained for samples that were normal in every respect and were not subjected to unusual environmental conditions during their growing periods. Geddes, Aitken, and Fisher (6) obtained a higher correlation coefficient between protein content and loaf volume than between any one farinogram characteristic and loaf volume. They concluded that the greatest utility of the farinograph was as a source of information supplementary to the baking test. Thus, the farinogram provided valuable information on correct absorption, optimum mixing time, and mixing tolerance. No data were presented, however, to indicate the degree of accuracy with which these variables could be predicted from the farinogram.

The mixograph has been used to demonstrate varietal differences among wheats that may or may not possess different baking qualities. Johnson, Swanson and Bayfield (10) have studied the relationships between loaf volume for normal samples and certain mixogram characteristics. They showed a highly significant relationship between loaf volume and curve height, width, and weakening angle. The relationships, however, were not as close as that between loaf volume and protein content. They concluded that the most important use of the mixograph is to furnish data that supplement the baking test. The mixograph provided information regarding mixing requirement, mixing tolerance, and varietal pattern. Mixing tolerance, as indicated by the rate of decrease in consistency or the weakening angle after a dough

had been mixed to its optimum consistency, was positively and significantly correlated with the decrease in loaf volume upon overmixing of the dough. While these workers indicated that mixograms could be used to predict mixing requirement, no data were presented.

The relationships between mixing time and protein content depend upon the instrument used. A positive linear correlation between these variables has been established for data obtained with the farinograph (1, 12, 13), while a curvilinear relationship exists between these variables for data obtained with the mixograph (3, 10).

A physicochemical test which has been suggested as a simple means for estimating protein quantity and quality is the sedimentation test (24, 25). It is a measure of the extent of hydration of the gluten. While it has been found that sedimentation value is related to protein content and loaf volume (24), no data are available to indicate its relationship to mixing characteristics. Since the mixing requirement of flour has been interpreted as a measure of the rate of hydration (22), it might be presumed that sedimentation value and mixing times are related. A marked advantage claimed by Zeleny (25) for the sedimentation test was that the inferior quality of wheats, such as ChiefKan, at any protein level could be detected. Schiller (21), however, reported difficulty in establishing a relationship between loaf volume and sedimentation value.

The object of this investigation was to study the relationship between physical characteristics measured by the farinograph and mixograph and to correlate curve factors measured by these instruments with loaf volume and experimental dough mixing time. Relationships between sedimentation value and other measures of quality also were studied.

Materials and Methods

The flours consisted of 60 samples representing five pure varieties of hard red winter wheat grown at twelve locations in Kansas in 1953–54 and 132 samples representing eight varieties of hard red winter wheat grown at seventeen locations in Kansas in 1954–55. The first series included the varieties Turkey, Pawnee, RedChief, Triumph, and Kiowa. The second series included the varieties Turkey, Ponca, Pawnee, Triumph, RedChief, Concho, C.I. 12518, and C.I. 12804. All wheat samples were milled to a straight-grade flour on an Allis mill (7). Data pertaining to these samples are presented in Tables I and II.

Moisture, ash, and protein were determined according to the methods of the A.A.C.C. (2). The straight-dough baking procedure was patterned after that described by Finney (4) and Finney and Barmore (5).

Baking absorption was based on farinograph data (500 B.U.). Duplicate doughs were scaled at 185 g. after fermentation of the dough made with 300 g. of flour. Experimental baking mixing times were determined by two experienced individuals by observation of the dough during mixing and without reference to farinograph or mixograph data. The criteria for optimum mixing time included extensibility, smoothness, and dryness of the dough. Mixing was stopped before the dough developed any undesirable degree of stickiness.

Mixograms were made according to the procedure of Johnson, Swanson, and Bayfield (10) and measured using the method described by Swanson and Johnson (23). The measurements included the time in minutes required to reach the peak of the curve and the dough weakening angle. Farinograms were made according to the method of Geddes *et al.* (6). The mixing tolerance index was calculated from farinograms according to the definition in *Cereal Laboratory Methods* (2). The valorimeter value was calculated according to the method described by Johnson *et al.* (8).

Sedimentation values were determined on the flour according to directions in *Cereal Laboratory Methods* (2).

Results and Discussion

Summaries of the analytical, baking, farinograph, mixograph, and sedimentation data for the 1954 and 1955 crop samples are presented in Tables I and II. The variety means were calculated for each variety grown at all stations. The station means were calculated for all varieties grown at a particular station. The minimum, maximum, and grand average for all samples are shown for each variable. The correlation analyses obtained through analyses of covariance for these samples are shown in Table III.

Relation of Farinograph and Mixograph Mixing Time to Experimental Baking Mixing Time. The experimental baking mixing time was highly correlated with mixograph mixing time from variety to variety among those varieties represented in this study (Table III). The correlation from location to location depended on season. It was highly significant for the 1955 crop but nonsignificant for the 1954 crop samples. Considering all varieties and locations together, there was a moderate positive correlation between experimental baking mixing time and mixograph mixing time (Table III and Fig. 1).

There was a positive but low total correlation between experimental baking mixing time and farinograph mixing time. None of the other correlations for these data were significant and all were generally lower than the corresponding correlations between experimental baking mix-

TABLE 1

SUMMARY OF VARIETY AND STATION AVERAGES FOR 1954 CROP SAMPLES

Variety and Station	Test Weight	Flour Yield	Flour Protein	Absorption	Exptl. Baking Mixing Time	Loaf Volume	Farinograph Mixing Time	Valorimeter Value	M.T.I. Units	Mixograph Mixing Time	Weakening Angle	Sedimentation Value
	lb.	*%*	*%*	*%*	*min.*	*cc.*	*min.*			*min.*	*deg.*	*ml.*
Variety means												
Turkey	60.4	70.3	12.4	69.3	4.7	825	8.6	72	19	3.3	13.7	56.2
Pawnee	62.1	69.7	12.8	69.3	3.1	848	4.9	55	39	2.1	18.6	42.8
RedChief	63.5	65.5	12.5	78.2	3.7	782	5.4	57	43	2.8	14.1	36.2
Triumph	62.6	69.6	13.4	68.9	3.3	841	5.5	59	37	2.1	21.5	55.8
Kiowa	62.2	70.2	13.8	71.5	3.3	865	7.0	65	26	2.7	17.8	51.2
Station means												
Columbus	63.9	71.5	12.2	69.8	4.0	799	5.1	56	46	2.2	23.7	44.6
Mound Valley	63.1	71.5	12.6	70.7	3.5	871	5.1	57	32	2.2	21.5	49.1
Thayer	63.6	71.7	11.5	68.2	3.7	791	5.4	58	38	2.2	20.4	40.9
Hutchinson	63.1	69.6	14.2	72.8	3.2	796	7.6	64	36	2.5	19.6	49.7
Canton	60.9	68.1	12.6	67.7	3.5	883	5.7	60	34	2.4	16.9	56.4
Manhattan	64.0	67.5	10.9	70.0	3.7	823	4.9	56	42	2.8	10.2	42.6
Belleville	62.3	69.0	12.0	68.7	3.3	887	5.2	57	42	2.6	12.3	44.6
Mankato	64.7	69.9	10.2	68.7	3.7	806	4.8	55	44	3.3	8.8	36.2
Hays	61.1	68.8	14.3	70.9	3.2	837	6.0	62	28	3.0	15.4	50.7
Dodge City	63.5	69.3	13.2	74.2	4.0	809	6.5	64	23	2.5	19.5	47.9
Tribune	57.8	66.5	16.1	77.7	4.0	797	9.9	76	16	2.5	21.2	59.7
Colby	57.8	65.4	15.8	78.0	4.0	887	8.8	73	13	2.8	16.1	58.8
Grand average	62.2	69.1	13.0	71.5	3.7	832	6.3	62	33	2.6	17.1	48.4
Minimum	56.0	61.7	9.1	64.0	2.3	635	3.0	46	0	1.6	6.0	31.2
Maximum	65.5	74.5	17.8	84.7	6.0	960	15.0	87	70	4.4	30.0	70.3

TABLE II

SUMMARY OF VARIETY AND STATION AVERAGES FOR 1955 CROP SAMPLES

VARIETY AND STATION	TEST WEIGHT	FLOUR YIELD	FLOUR PROTEIN	ABSORPTION	EXPTL. BAKING MIXING TIME	LOAF VOLUME	FARINOGRAPH MIXING TIME	VALORIMETER VALUE	M.T.I. UNITS	MIXOGRAPH MIXING TIME	WEAKENING ANGLE	SEDIMENTATION VALUE
	lb.	*%*	*%*	*%*	*min.*	*cc.*	*min.*			*min.*	*deg.*	*ml.*
Variety means												
Turkey	59.6	72.3	12.1	64.9	3.4	819	4.9	55	35	2.6	17.0	45
Ponca	60.2	69.0	12.5	64.2	3.8	839	4.1	58	36	3.2	15.7	46
Pawnee	60.0	72.0	12.3	64.4	3.0	805	4.0	50	52	2.2	22.0	40
Triumph	61.2	69.2	12.1	64.1	3.5	796	4.6	53	44	2.5	22.6	47
RedChief	62.5	70.2	12.0	67.6	3.0	780	3.6	48	54	2.4	19.4	34
Concho	60.4	68.8	12.0	66.8	3.4	806	4.6	55	39	2.5	19.1	43
C.I.12518	60.2	71.8	12.8	66.1	4.2	849	5.9	60	36	3.0	18.0	49
C.I.12804	60.5	68.3	12.3	67.4	3.5	853	4.7	54	44	2.7	16.8	46
Station means												
Manhattan	60.1	68.2	14.3	64.3	3.8	969	5.1	57	29	2.8	19.1	59.5
Hays	63.1	68.5	12.4	65.3	3.1	938	4.6	56	37	2.7	15.5	46.0
Colby	62.7	66.0	11.4	65.1	2.8	864	3.4	47	59	2.3	20.0	32.7
Tribune	61.5	59.1	11.9	62.3	2.9	782	3.3	46	58	2.0	28.1	34.8
Garden City	61.9	69.5	13.5	66.1	2.6	749	3.8	48	54	2.0	27.4	39.2
Garden City[a]	63.2	71.2	10.9	65.0	2.3	677	2.6	40	84	1.8	24.1	28.7
Mound Valley	58.5	73.3	11.2	63.4	4.6	833	4.6	56	36	3.5	8.0	44.3
Thayer	60.2	70.5	11.0	63.3	5.1	830	5.0	58	35	3.8	10.8	42.7
Columbus	61.2	72.9	10.6	63.1	4.0	854	4.5	54	45	2.8	14.3	39.2
Belleville	62.5	71.4	13.3	69.1	3.0	746	5.0	57	32	2.6	20.3	46.2
Mankato	61.5	72.0	13.7	69.6	3.1	748	5.7	61	26	3.0	21.1	53.5
Concordia[a]	63.7	69.7	10.1	64.5	2.8	721	4.0	51	50	2.3	18.3	29.4
Dodge City	52.1	66.5	13.5	66.5	3.1	919	5.5	61	28	2.6	17.8	51.9
Hutchinson	51.9	64.3	14.9	67.0	3.1	927	6.6	65	27	2.7	20.3	58.4
Canton	62.4	73.2	15.3	70.0	3.3	873	8.4	73	19	2.8	25.9	64.2
St. John	61.4	74.0	12.0	69.7	2.6	779	4.4	52	46	2.4	19.6	42.8
Hiawatha	61.0	77.7	8.4	63.8	3.2	692	1.7	39	61	2.8	10.8	27.8
Grand average	60.5	69.9	12.3	65.8	3.3	823	4.6	54	43	2.6	18.9	44.0
Minimum	50.2	46.0	8.1	59.7	1.8	640	1.0	30	10	1.0	4.0	23.0
Maximum	64.3	82.0	16.5	75.4	7.5	1000	10.5	78	100	5.0	42.0	72.5

[a] Irrigated.

TABLE III

SUMMARY OF CORRELATION ANALYSES THROUGH ANALYSES OF COVARIANCE FOR SAMPLES GROWN IN TWO CROP YEARS

VARIABLES CORRELATED	TYPE OF CORRELATION			
	VARIETAL	LOCATIONAL	INTERACTION	TOTAL
Data for 1954	Degrees of freedom			
	3	10	44	58
Baking mixing time *vs.* farinograph mixing time	0.818	0.334	0.124	0.450***
Baking mixing time *vs.* mixograph mixing time	0.892*	−0.072	0.489***	0.585***
Loaf volume *vs.* flour protein	0.740	0.110	0.191	0.189
Loaf volume *vs.* valorimeter value	0.197	−0.001	0.012	0.056
Mixing tolerance index *vs.* weakening angle	0.305	−0.277	0.540***	0.101
Valorimeter value *vs.* sedimentation value	0.685	0.817**	0.357*	0.665***
Baking mixing time *vs.* sedimentation value	0.335	0.124	−0.446**	0.065
Flour protein *vs.* sedimentation value	0.384	0.883***	0.445**	0.607***
Loaf volume *vs.* sedimentation value	0.565	0.380	0.174	0.348**
Data for 1955	Degrees of freedom			
	6	15	107	130
Baking mixing time *vs.* farinograph mixing time	0.488	0.261	−0.128	0.273**
Baking mixing time *vs.* mixograph mixing time	0.836**	0.892***	0.399***	0.772***
Loaf volume *vs.* flour protein	0.736*	0.517*	0.078	0.467***
Loaf volume *vs.* valorimeter value	0.749*	0.592**	0.347***	0.568***
Mixing tolerance index *vs.* weakening angle	0.622	0.145	0.040	0.178*
Valorimeter value *vs.* sedimentation value	0.856**	0.906***	0.351***	0.836***
Baking mixing time *vs.* sedimentation value	0.508	0.249	0.006	0.256**
Flour protein *vs.* sedimentation value	0.551	0.876***	0.465***	0.749***
Loaf volume *vs.* sedimentation value	0.674*	0.656**	0.391***	0.616***

ing time and mixograph mixing time. It is obvious that the farinograph mixing time did not provide as accurate an estimate of experimental baking mixing time as did the mixograph mixing time. The relationship between farinograph and mixograph mixing time was of low magnitude. This was shown by the total correlation coefficient of 0.52 for the 1955 crop year (Fig. 1). The suggested use of the mixograph as a means of segregating wheats on the basis of mixing characteristics prior to binning apparently is sound (9, 17).

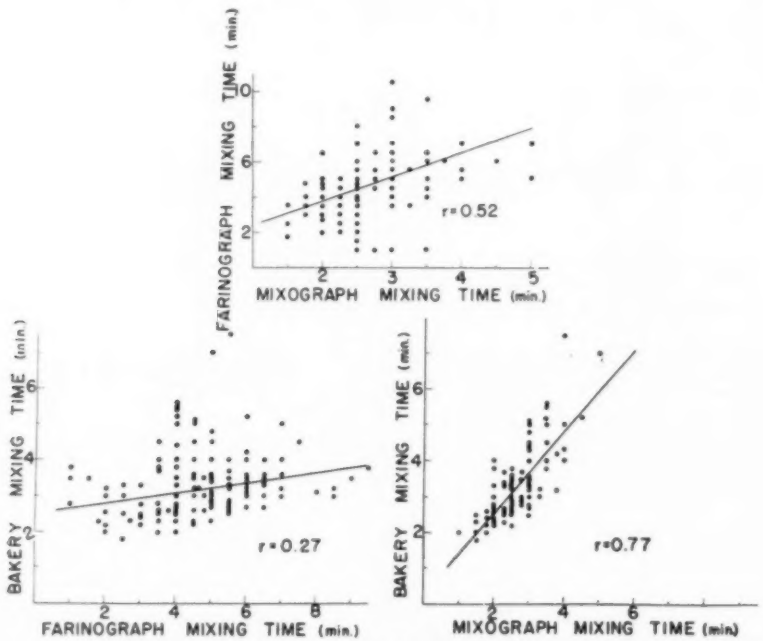

Fig. 1. Relationships among farinograph, mixograph, and experimental baking mixing times for 1955 crop flours.

The reasons are not known why the farinograph and mixograph mixing times are not more highly correlated or why the mixograph mixing time is more closely related to the experimental baking mixing time than is the farinograph mixing time. It may be that the high-speed types of mixing in the mixograph and bakery mixes are sufficiently alike to produce similar results, whereas the farinograph type of mixing is slow and gentle and unlike that of American-type mixers. It is possible that the farinograph mixing time is correlated to a higher degree with the baking mixing time obtained using European type

mixers. It would appear worthwhile to study the correlation of mixograph and farinograph mixing times with the mixing time required for the same flours in commercial shops. It would be difficult to obtain high correlations, however, because flours for bakery use are relatively uniform and represent a narrow range of mixing times.

Relation of Flour Protein and Loaf Volume and of the Valorimeter Value to Loaf Volume and Flour Protein. None of the four correlations between loaf volume and flour protein (Table III) was significant for the 1954 crop samples. All except the interaction correlation were significant for the 1955 crop samples when the sampling was on a larger scale. There was a moderate positive correlation between loaf volume and protein content from variety to variety. The nonsignificant correlation (0.19) for the relationship between protein content and loaf volume for the 1954 samples suggested that these samples were of inferior quality. The same relationships for the 1955 crop samples, excepting the interaction, although only moderately high, were significant indicating that the 1955 samples were nearer normal than the 1954 samples.

The scatter diagram for the relationship between loaf volume and protein content for the 1955 samples is shown in Fig. 2. In view of the high correlations between flour protein and loaf volume reported in the literature (5, 10, 11) it is concluded that the quality of the samples represented in this study was inferior. This probably was due to unusual environmental conditions during the ripening periods.

The loaf volume and valorimeter data (Table III) were not significantly correlated for the 1954 crop samples. All four of the correlations were significant and moderate in size for the 1955 crop samples. The correlation between valorimeter values and flour protein data for the 1955 crop samples was significant. A scatter diagram of the data is shown in Fig. 2.

Relation of Mixing Tolerance Index and Weakening Angle. The correlations between mixing tolerance index and weakening angle were generally nonsignificant except for the interaction for the 1954 crop samples and the total for the 1955 crop samples. Although it might be expected that these two measurements evaluate the same general flour characteristic, the data in Table III and Fig. 3 indicate no significant relationship. Since it has been established that the weakening angle of the mixograms gives a reasonable measure of the sensitivity of a flour to mixing in the laboratory (10), it must be concluded that the mixing tolerance index is not an adequate measure of the sensitivity of a flour to mixing.

Relations of Sedimentation Value to Protein, Valorimeter Value,

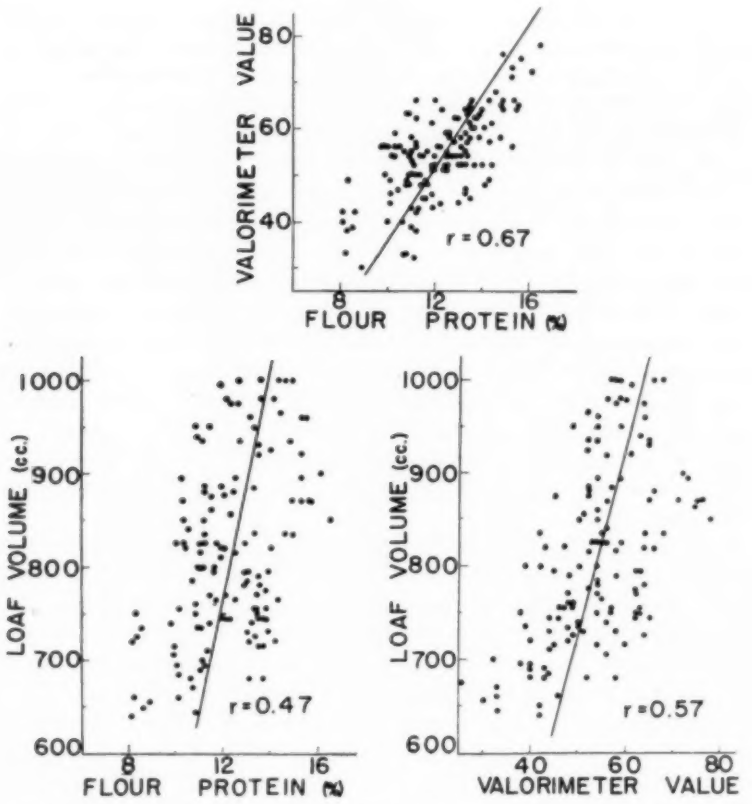

Fig. 2. Relationships between flour protein and loaf volume, valorimeter value and loaf volume, and valorimeter value and flour protein for 1955 crop flours.

Baking Mixing Time, and Loaf Volume. The relationships between experimental baking mixing time and sedimentation were not highly correlated. For the 1954 samples, the interaction of variety and location was significant. For the 1955 samples, the interaction, variety, and location correlations were nonsignificant, although the total correlation was significant at the 1% level. A plot of the 1955 data is shown in Fig. 4.

Protein and sedimentation value were positively and strongly correlated with respect to location and with respect to the interaction between location and variety. There also was a significant total correlation between these variables (Fig. 4). Variety did not account for a significant part of the correlation between these variables. Significance

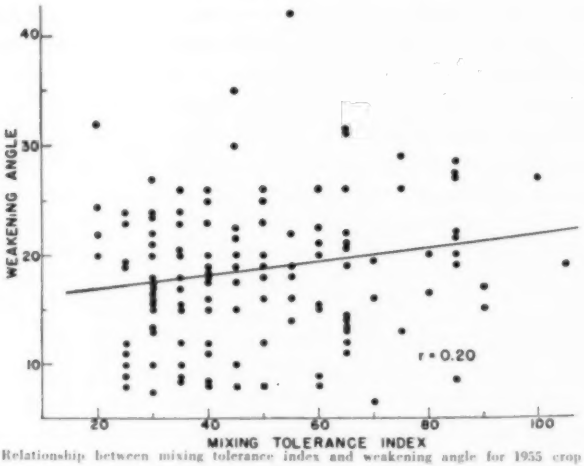

Fig. 3. Relationship between mixing tolerance index and weakening angle for 1955 crop flours.

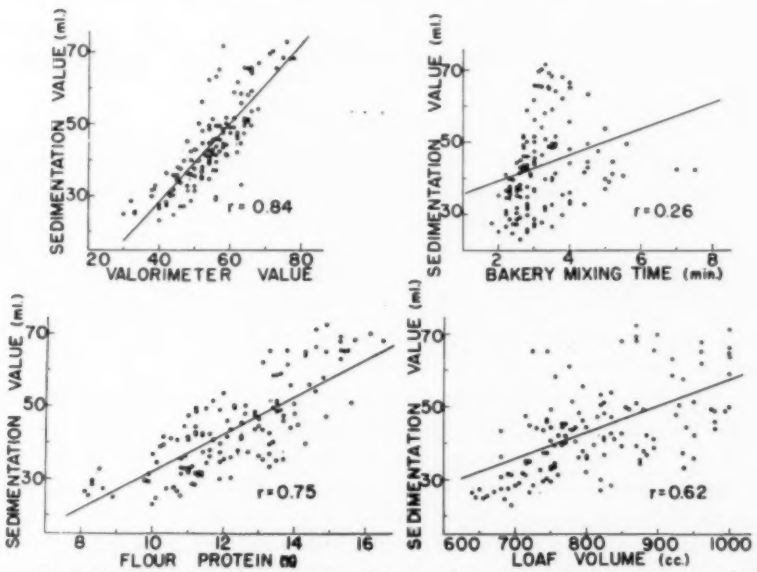

Fig. 4. Relationships between flour protein, valorimeter value, experimental baking mixing time, loaf volume, and sedimentation value for 1955 crop flours.

might have been attained if a larger number of varieties had been employed.

Valorimeter and sedimentation values probably are positively correlated from variety to variety although this correlation was nonsig-

nificant in 1954. There was a strong positive correlation between valorimeter and sedimentation values from location to location. The interaction of variety and location was weak. The total correlation ranged from moderate to strong, depending on season. A plot of the data for the 1955 crop is shown in Fig. 4.

Loaf volume and sedimentation value were not significantly correlated for the 1954 crop samples except for the total correlation which was weak. All four correlations were significant but moderate in size for the 1955 crop samples. A plot of these data also is shown in Fig. 4.

The correlation coefficient of 0.75 between sedimentation value and flour protein for the 1955 samples corresponds well with the value of 0.79 reported by Zeleny (24) for 185 samples, but the correlation coefficient of 0.62 between sedimentation value and loaf volume is much less than that (0.863) reported (24).

Since the correlation coefficient between sedimentation value and loaf volume (0.62) for the 1955 crop was higher than the correlation between flour protein and loaf volume (0.47), it must be assumed that the sedimentation test measures part of the protein quality differences among different flours. Similar differences existed for the 1954 crop data.

General Discussion

The various correlation coefficients calculated for the 1955 crop samples are generally higher than for corresponding variables calculated for the 1954 series. Since environment during the ripening period of a wheat is known to have an important bearing on the baking properties of its flour (20), it is possible that the adverse effects of environment on the 1954 and 1955 crop samples caused them to have more abnormal mixing and baking properties than for the samples reported in the literature (5, 10, 11).

Although the samples tested may have been abnormal, it is believed that any valid test for quality should reveal quality differences. As an aid to the reader, several of the scatter diagrams for the 1955 crop data are shown in Figs. 1 to 4. Although all of the relationships studied were highly significant for the 1955 crop samples, only three were sufficiently correlated to be of some value for prediction. These were baking and mixograph mixing time, flour protein and sedimentation value, and sedimentation value and valorimeter value. These relationships should be useful to those interested in rapid and simple tests to measure flour quality. The mixograph would appear to be more useful in predicting baking mixing time than the farinograph. The data also suggest that the weakening angle from mixograms is a better measure

of the sensitivity of a flour to mixing than is the mixing tolerance index taken from farinograms.

Acknowledgments

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DECOMPOSITION OF BROMATE IN FERMENTING AND NONFERMENTING DOUGHS[1]

D. K. CUNNINGHAM AND J. A. ANDERSON

ABSTRACT

In both fermenting and nonfermenting doughs mixed in nitrogen, a small initial loss of bromate is followed by a continuing loss at uniform rate. The rate of the continuing loss, of the order of 1 to 3.5 p.p.m. per hour, is increased by increasing initial bromate level and by decreasing flour grade. The rate was lower in fermenting than in nonfermenting doughs for five clear and two other low-grade flours, whereas the reverse held for two patent flours. Fermenting doughs mixed in air showed a lower rate than those mixed in nitrogen, and a still lower rate when punched and molded. The small initial loss (0.2 to 3.5 p.p.m.) was also greater for higher initial bromate levels. The correlation coefficient for nine flours between rate of continuing bromate loss (nonfermented doughs mixed in nitrogen) and ash content was $r = 0.91^{**}$.

While the changes produced by potassium bromate in the physical properties of doughs have been widely studied, little is yet known about the chemical reactions that cause these changes. A historical review of investigations in this field has recently been published by Aitken (1). For more than twenty years it has been held, as a general hypothesis, that the changing properties of doughs are due mainly to alterations in the complexity of a protein network. More detailed hypotheses have postulated that bromate affects the network either indirectly by inhibiting proteases or directly by affecting sulfur linkages in the protein. In addition, Cookson and Coppock (3) have recently suggested that lipids play a significant part in the mechanism of oxidative improvement. Perhaps it is reasonable to say that none of these

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hypotheses has been clearly eliminated nor adequately confirmed; the situation is complex and remains obscure.

One line of attack that may aid in solving this problem involves study of the rates of disappearance of bromate from dough under various conditions. Data on this point were obtained in 1940 by Sullivan, Howe, Schmalz, and Astleford (14). They found that over half the bromate in a straight dough was used immediately upon mixing, that a comparatively small amount was used during the 3 hours of fermentation, and that no tests could be obtained for bromate when the bread was taken from the oven. A more detailed study with unfermented and fermented doughs was subsequently undertaken by Hlynka, Templin, and Anderson (10) in 1953. Literature on the disappearance of bromate, iodate, and chlorite was reviewed. Their studies showed little or no loss of bromate in unfermented doughs mixed up to 8 minutes and then subjected to various manipulations during the following 3 hours. Yeast was found to accelerate the decomposition of bromate, and progressive disappearance of bromate was reported at various stages of the test baking procedure. With the unfermented doughs in particular, the lack of correlation between bromate disappearance and the effect of the reagent on the physical properties of the dough was especially noteworthy.

During the latter study it was found that the errors for available bromate methods (11, 12) were considerably greater for dough than for flour. Moreover, analytical error appeared to be a limiting factor in interpreting results. Accordingly, attention was turned to improvement of procedures for determining bromate, and an amperometric method for bromate (4) in flour was developed. This procedure has now been adapted to dough and used in a further study of the rates of bromate decomposition in fermented and unfermented doughs made from flours of different grades. The principal studies deal with doughs that were mixed in nitrogen to avoid the interfering effects of oxygen of the air.

Method

Reagents. 1) Zinc sulfate, 155.1 g. per liter; 2) sodium hydroxide, 26.0 g/l; 3) n-octyl alcohol; 4) sodium thiosulfate, 0.001 *N*; 5) potassium iodate, 0.001 *N*; 6) potassium iodide, 30%; 7) sulfuric acid, 10%.

Procedure. Doughs were mixed in nitrogen (or in air) for 2.5 minutes in the G.R.L. mixer (9), and stored at 30°C. and 85% relative humidity. Forty-gram subsamples of dough were weighed out at the required times and were dispersed in 181.5 ml. of water in a two-speed Waring Blendor for 3 minutes at low speed. In this time, 25 ml.

of zinc sulfate reagent and 25 ml. of sodium hydroxide reagent were added with a few drops of n-octyl alcohol. The blender was then operated at high speed for a few seconds and the mixture was centrifuged at a relative centrifugal force of 1500 G for 10 minutes. By this procedure floating scum was eliminated and crystal-clear extracts were obtained. Bromate was determined in 20 ml. of the extract by adding 5 ml. of 0.001 *N* sodium thiosulfate solution, 5 ml. of 10% sulfuric acid, and 3 ml. of 30% potassium iodide solution and back-titrating with 0.001 *N* potassium iodate solution. The end-point was determined amperometrically and bromate was calculated on a 14% moisture basis, taking into account water contributed by the ingredients and by the flour.

Accuracy. The method was tested for estimation of bromate in dough from an experimentally milled flour (ash, 0.43%; protein, 11.9%). The dough was mixed under nitrogen to contain 1% salt and 10.0, 12.5, 15.0, 20.0, 25.0, 27.5, 37.5, 40.0, 93.8, and 187.5 p.p.m. of potassium bromate. Average recovery was 98.53% (46.2 p.p.m.) and the standard error was ±1.9% (±0.9 p.p.m.).

Bromate Recovery in Nonfermented Doughs Mixed in Nitrogen

Studies were first made of bromate recoveries from nonfermented doughs. The four test flours (A, B, C, D, Table I) were untreated commercial mill fractions from hard red spring wheat. Doughs were mixed in nitrogen to contain 1% salt, 15 and 30 p.p.m. of potassium bromate, and water to the baking absorption. The doughs were stored in a cabinet at 30°C. and 85% R.H. Bromate was determined immediately after mixing and at hourly intervals for 4 hours.

In Fig. 1, bromate recoveries are plotted against time. These curves show that loss of bromate increases with time for all flours. Bromate appears to disappear most rapidly from the low-grade flours C and D. The data may be conveniently summarized by calculating the line of best fit for recoveries against time (omitting the zero time recovery), when the slopes of these lines (Table I) represent rates of loss. For both levels of bromate treatment, the rate of loss increased in the order A, B, D, C, indicating that rate of bromate decomposition is greater in lower-grade flours.

The data in Table I also show that the rate of bromate loss increases with the initial bromate level. Additional data on this point were obtained for initial bromate levels of 10, 20, and 25 p.p.m. However, these determinations were made 10 months later than those for 15 and 30 p.p.m. levels, and it was evident that changes had taken place in the flours during this period of storage. Rates of loss of bro-

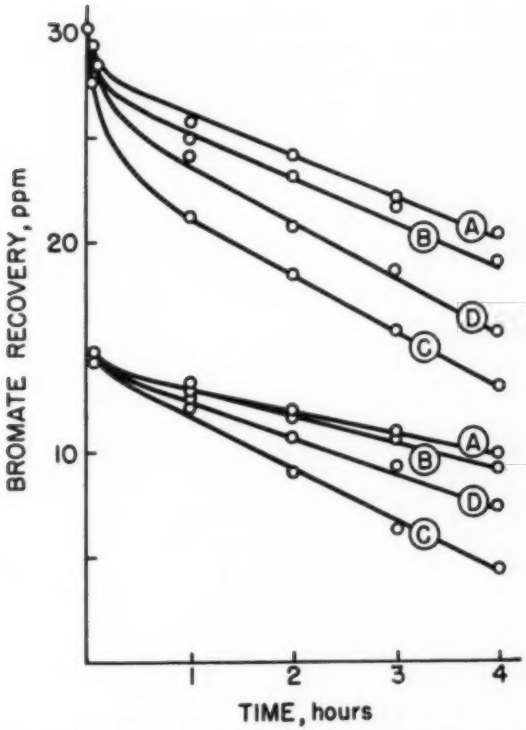

Fig. 1. Bromate recoveries during 4 hours with initial levels of 15 and 30 p.p.m. of potassium bromate from four flours: A, patent; B, intermediate; C, germ; and D, clear flour.

mate remained much the same for the flours A and D (patent and clear) but decreased greatly for flours B and C (intermediate and germ), for which experiments were repeated at levels of 15 and 30 p.p.m. The data are shown graphically in Fig. 2, in which the rate of loss is plotted against initial bromate concentration. The effects of

TABLE I

RATES OF BROMATE LOSS (P.P.M. PER HOUR) IN FOUR FLOURS AT TWO INITIAL BROMATE LEVELS

FLOUR	PROTEIN (N × 5.7)	ASH	BROMATE LOSS	
			15 p.p.m.	30 p.p.m.
	%	%		
A. Patent	12.4	0.40	1.07	1.28
B. Intermediate	13.0	0.56	1.60	2.46
C. Germ	12.7	0.96	2.82	3.01
D. Clear	15.2	0.92	1.86	2.71

storage on flours B and C probably represent changes in the flours caused by reaction with atmospheric oxygen during the storage period. It is clear, however, that the rate of bromate loss in each flour increases directly with the initial level of bromate applied.

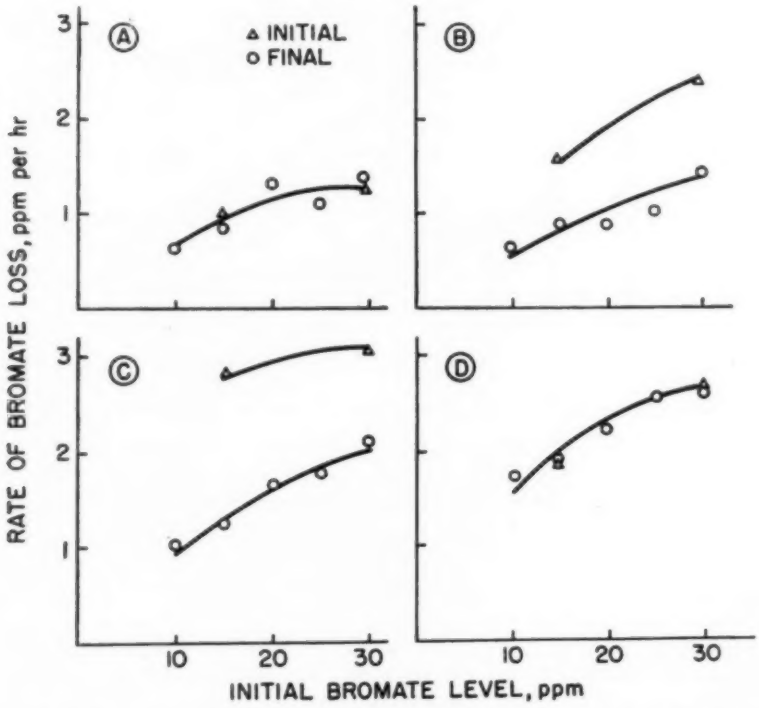

Fig. 2. Rate of bromate loss for flours tested initially and 10 months later with 10, 15, 20, 25, and 30 p.p.m. of potassium bromate: A, patent; B, intermediate; C, germ; and D, clear flour.

The curves in Fig. 1 also suggest that bromate is lost more rapidly during the first hour than thereafter. If it is assumed, as seems probable, that this early loss occurs during mixing, its amount can be estimated by extrapolating the line of best fit to zero time. The intercept is less than the initial bromate level, and the difference represents the initial loss of bromate. Initial losses, thus calculated, are given in Table II for the four flours after storage. The data suggest that the apparent initial loss increases with increasing level of bromate treatment. Differences between flours are not sufficiently consistent to permit reliable conclusions.

TABLE II

APPARENT INITIAL LOSS OF BROMATE IN FLOURS A, B, C, D, AT FIVE BROMATE LEVELS

FLOUR	INITIAL BROMATE LEVEL (P.P.M.)				
	10	15	20	25	30
A. Patent	+0.2	+1.0	+0.2	+2.8	+2.5
B. Intermediate	+0.5	+0.2	+0.7	+1.6	+2.3
C. Germ	+0.5	+1.4	+0.6	+3.2	+3.8
D. Clear	0.0	+1.0	+0.9	+1.6	+3.5

Bromate Recovery from Fermented Doughs

In the above work, bromate recoveries were determined in non-fermenting doughs mixed in nitrogen; these typified doughs used in this laboratory for investigation of physical properties. However, the commercial use of potassium bromate is in fermenting doughs, which are mixed in air and subjected to punching, panning, and proofing. A second series of studies was designed to compare rates of bromate loss in 1) fermented doughs mixed in nitrogen; 2) fermented doughs mixed in air; and 3) fermented doughs mixed in air, and punched, panned, and proofed.

For each of the flours A, B, C, and D, doughs were mixed to the basic A.A.C.C. malt-phosphate formula at 15 p.p.m. of bromate. Sets 1 and 2, mixed in nitrogen and in air, were allowed to rest undisturbed at 30°C., except for sampling immediately after mixing and at hourly intervals thereafter for 4 hours. To evaluate the effect of working the dough on rate of bromate loss, a series of five identical doughs was prepared for each flour (set 3). The first dough was sampled and residual bromate was determined at mixing, the second after the first punch, the third after the second punch, the fourth after panning, and the fifth after proofing. The data are summarized in Table III by means of rates of loss representing the slopes of the lines of best fit.

The data in Table III indicate that mixing in air decreases the rate of loss of bromate compared to mixing in nitrogen, though the

TABLE III

RATE OF LOSS (P.P.M. PER HOUR) OF POTASSIUM BROMATE (INITIALLY 15 P.P.M.) IN FERMENTED DOUGHS

FLOUR	MIXED IN NITROGEN	MIXED IN AIR	MIXED IN AIR AND WORKED
A. Patent	1.18	1.16	0.95
D. Clear	1.40	0.93	0.88
B. Intermediate	1.46	0.98	0.60
C. Germ	2.01	1.76	1.29

decrease is very small for the patent flour A. When the doughs are worked, the rate of loss is reduced still further, especially for flours B and C. Moreover, whereas the rates are in increasing order for patent, clear, intermediate, and germ flours, when these are mixed in nitrogen, the clear and intermediate flours show the lowest rates when mixed in air or worked. It seems probable that the decreases in rate when doughs are mixed in air or worked are the result of competition between bromate and oxygen for oxidizable constituents of the dough. The situation is obviously complex, since changing from nitrogen to air, and working the dough, have a greater effect on some flours than on others.

Comparison of Nonfermented and Fermented Doughs

Since potassium bromate is known to affect the physical properties of fermenting as well as nonfermenting doughs (1), the rates of loss of bromate in fermenting and nonfermenting doughs were compared. The necessary data were available for flours A, B, C, and D mixed in nitrogen at initial levels of 15 and 30 p.p.m. of potassium bromate; in order to extend the investigation, rates of bromate loss were determined in the same manner for an initial level of 30 p.p.m. bromate on a second series of flours (E, F, G, H, and I, Table IV).

Data for all nine flours are shown in Table IV, with flours listed in order of increasing rate of loss for the nonfermented doughs at an initial level of 30 p.p.m. The rates indicate that fermentation decreases bromate loss in doughs mixed initially in nitrogen, except for the two patent flours (A and E) for which rate of loss is increased. While these conclusions are based on the 30 p.p.m. initial level, the same generalizations hold at the 15 p.p.m. level for the four flours for which data are available. It thus appears that yeast and bromate may compete for reducible substances introduced by the germ and bran constituents in lower grade flours.

Relation of Rate of Bromate Loss to Flour Composition

The rate of bromate loss in nonfermented doughs mixed in nitrogen was shown to increase with decreasing grade of flour for the first series of flours, and also for the second series. Correlation coefficients for nine pairs of data ($n = 7$) were computed for rate of loss with the protein, ash, and fat (petroleum ether-soluble).

Correlation	r
Rate of bromate loss × ash	0.91**
Rate of bromate loss × lipid	0.78**
Rate of bromate loss × protein	0.59*

These coefficients show that rate of bromate loss is significantly and positively correlated with ash content, and with fat content, but that

TABLE IV

RATE OF BROMATE LOSS (P.P.M. PER HOUR) IN NONFERMENTED AND FERMENTED DOUGHS

FLOUR	PROTEIN (N × 5.7)	ASH	INITIAL BROMATE LEVEL			
			15 p.p.m.		30 p.p.m.	
			NONFERMENTED	FERMENTED	NONFERMENTED	FERMENTED
	%	%				
A. Patent	12.4	0.40	1.07	1.18	0.99	1.79
E. Patent	12.5	0.37	.....	.....	2.10	2.26
B. Intermediate[a]	13.0	0.56	1.60	1.46	2.46	1.90
F. Clear	22.2	0.86	.....	.....	2.84	2.51
C. Germ	12.7	0.96	2.82	2.01	3.01	2.81
D. Clear	15.2	0.92	1.86	1.40	3.07	2.56
G. Clear	15.3	0.90	.....	.....	3.37	2.60
H. Clear	15.9	1.05	.....	.....	3.96	3.19
I. Clear	20.9	1.27	.....	.....	4.50	3.14

[a] A flour fraction excluding high-grade middling and clears.

the correlation with protein content was not significant. It should be mentioned that the correlation between rate of bromate loss and fat content is probably incidental, since the partial correlation for these two properties independent of ash content was, $r_{lf.a} = 0.13$.

Discussion

The *intra*-flour relations between bromate loss and the effects of bromate on dough properties and baking behavior are relatively simple. Within each flour, the main loss of bromate in nonfermenting dough, mixed in nitrogen, occurs at a uniform rate of the order 1 to 3.5 p.p.m. per hour. This finding is at variance with earlier results (10) obtained in this laboratory with less precise methods. The bromate loss may be said to parallel the change in physical properties (e.g. relaxation constants) of similarly treated dough that also proceed at essentially constant rate (5, 6). Moreover, within any one flour, an increase in the initial concentration of bromate causes an increase in the rate of bromate loss and a corresponding increase in the rate of change of the relaxation constant. Increasing bromate concentration also increases the baking response; for, in this sense, the decrease in loaf volume that occurs after the optimum bromate level has been exceeded is no less a bromate response than the prior increase in loaf volume.

The corresponding *inter*-flour relations are more complex. Rheological and baking data for four of the flours used in this study were reported in an earlier paper (5). Rates of change of relaxation constants (5, Fig. 3) were in the following rank order: clear, B; intermedi-

decrease is very small for the patent flour A. When the doughs are worked, the rate of loss is reduced still further, especially for flours B and C. Moreover, whereas the rates are in increasing order for patent, clear, intermediate, and germ flours, when these are mixed in nitrogen, the clear and intermediate flours show the lowest rates when mixed in air or worked. It seems probable that the decreases in rate when doughs are mixed in air or worked are the result of competition between bromate and oxygen for oxidizable constituents of the dough. The situation is obviously complex, since changing from nitrogen to air, and working the dough, have a greater effect on some flours than on others.

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ate, C; patent, A; and germ, D. For the first three of these flours, the rates of change of relaxation constant and the rates of bromate loss (both determined in unleavened doughs mixed in nitrogen) are in the same rank order.

On the other hand, the germ flour, which shows the greatest rate of bromate loss. shows the lowest rate of change of physical properties. If it is assumed that the effect of bromate on physical properties of doughs is a function of its oxidizing activity, and thus of its rate of loss in the dough, the simplest explanation of the anomalous position of the germ flour appears to be as follows: It may be postulated that, in germ flour, bromate is involved in two types of reaction; that only one of these affects the physical properties of the dough (if its rate could be measured separately, the germ flour might well fall in its proper rank order); and that the second reaction involves reducing constituents that play no role in altering the physical properties of the dough. This also may be true, though to a lesser extent, for the other flours used in this study.

The inter-flour relations involving bromate loss and baking behavior present a more difficult problem in theoretical elucidation. Comparisons must be made with doughs mixed in oxygen and subsequently worked by punching and molding. Both bromate (5, 14) and oxygen (2, 7, 8, 13) affect the baking response and may well compete for the same reducing constituents in some flours but not in others (e.g. in the germ fraction). In the face of these complications, further speculation seems premature.

Our conclusions are that the results of the present study are consistent with the hypothesis that the "improving" action of bromate, as shown by changes in physical properties of the dough or by baking response, is a function of the oxidizing action of potassium bromate. Nevertheless, though consistent with it, the data provide little verification for the hypothesis.

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INVESTIGATIONS OF THE WATER-SOLUBLE NITROGENOUS FRACTION OF RICE[1]

Russell T. McIntyre

ABSTRACT

Electrophoresis of unpurified aqueous extracts of brown rice indicated that the albumins were heterogeneous. No discrete protein fraction was observed to migrate at pH 4.0, or 8.4, or 10.3. Partial purification of the extract by ammonium sulfate fractionation did not improve its electrophoretic pattern at pH 10.3.

The nonprotein nitrogen content of the aqueous extract was found to be 36% of the total water-soluble nitrogen. This value corresponds to 3.5% of the total nitrogen content of the rice.

One of the earliest investigations of the proteins in rice indicated the presence of four proteins as based on the classical solubility scheme (5). These included glutelin, prolamine, globulin, and albumin fractions. Jones and Gersdorff (2), some two decades later, were unable to verify the presence of an albumin in white rice. Rice bran, however, was found to contain an albumin corresponding to 0.13% of the bran. Moreover, fractional coagulation procedures indicated two components were present in this water-soluble fraction.

[1] Manuscript received January 9, 1956. Contribution from the Louisiana Agricultural Experiment Station, Baton Rouge.

Sturgis, Miears, and Walker (6) found the nitrogen contained in the water-soluble fraction of brown rice varies from about 6 to 11.5% of the total nitrogen content (29 varieties and selections grown in Louisiana). No attempt was made to determine the amount of nonprotein nitrogen in the water-soluble fraction. Jodidi (1) observed that the nonprotein nitrogen content of three varieties varied from 4 to 5% of the total nitrogen as determined by phosphotungstic acid precipitation of proteinaceous constituents.

The use of electrophoresis in the separation of mixtures of proteins is well known. In the present study this method was applied to aqueous extracts prepared from brown rice to determine the electrokinetic nature of the proteins. The nonprotein nitrogen was also determined.

Materials and Methods

Brown rice (Rexoro) was used in the studies. On a dry-weight basis the nitrogen content was 1.58% (9.40% protein). The rice was ground in a laboratory Wiley mill equipped with a 60-mesh screen. To remove the fat, a 20-g. sample was stirred into 40 ml. of ether, and after 30 minutes the ether was decanted. This procedure was repeated three times. After the final extraction the rice was allowed to stand in the hood 2 days in a shallow dish.

Preparation of Samples for Electrophoresis. Twenty grams of defatted rice were placed in a small beaker and mixed with 20 ml. of water. After this had stood 1 hour with intermittent stirring, the liquid was filtered off and the cake was washed with water until the filtrate volume was 20 to 25 ml.

Before electrophoresis, the sample was placed in cellophane tubing and dialyzed for 48 hours at 2°C. against the appropriate buffer. At least four changes of buffer were employed during this equilibration period. Buffers having an ionic strength of 0.1 were prepared as described by Miller and Golder (3). Since a small amount of protein precipitated from solution during dialysis, the samples were filtered (Whatman No. 1) prior to electrophoresis.

Electrophoresis. Electrophoresis was carried out with the Klett electrophoresis apparatus. The standard double-length cell of the Tiselius type (11 ml.) was employed. A current of 10 ma. was used. The time of electrophoresis was between 4 and 5 hours.

Nonprotein Nitrogen Determinations. Ten grams of ground rice were allowed to stand 1 hour with 40 ml. of water (10°C.), the liquid was filtered off, and the cake washed with 10 ml. of water. The filter paper and residue were transferred to a glass-stoppered flask and allowed to stand overnight with 30 ml. of water (2°C.). The mixture

was filtered and the cake washed with water until the volume of the combined filtrates was 100 ml.

To determine the nonprotein nitrogen, 5 ml. of the filtrate were treated with 1 ml. of 20% trichloracetic acid. After standing a short time, the solution was centrifuged and an aliquot of the supernatant removed for nitrogen analysis.

Nitrogen was determined by a micro-Kjeldahl method using copper sulfate-potassium sulfate catalyst. Distillation was carried out with a Pregl type micro-Kjeldahl steam distillation apparatus.

Results and Discussion

Electrophoretic analysis of the aqueous extracts indicate that the protein constituents are grossly inhomogeneous (Fig. 1). The majority of the protein appears to be included in the faster-moving portion of the protein. This portion, however, exhibits strong spreading and does not indicate the presence of a single discrete protein. At pH 10.3, a small component was observed to migrate in the opposite direction from the rest of the protein. Partial purification by fractionation (25 to 50% saturation with ammonium sulfate) did not markedly improve the sharpness of the peaks when the protein boundary was allowed to migrate at pH 10.3 (pattern not shown). From the work of Jones and Gersdorff (2) it was anticipated that at least two main com-

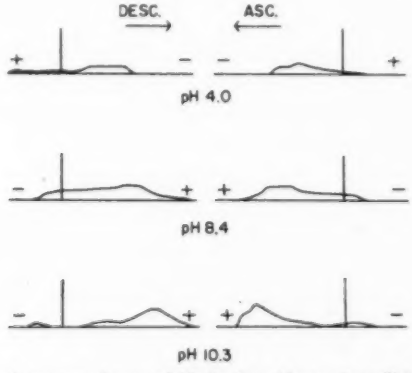

Fig. 1. Electrophoretic patterns of water-soluble fraction of brown rice: pH 4.0, acetate buffer, current 10.12 ma., 4.7 hours; pH 8.4, phosphate buffer, current 10.28 ma., 4.3 hours; pH 10.3, glycine buffer, current 10.21 ma., 5.0 hours. The vertical line represents the position of the initial boundary.

ponents would be separated from the brown rice extract. This was not observed. The results do not necessarily mean that at least two fractions are not present. These proteins could have the same electrophoretic mobility or their presence could be "covered up" by the

electrophoretic behavior of other components. The protein preparations gave a negative test for xylose-containing carbohydrates.

It is possible that heterogeneity is characteristic of the albumins of cereal grains. Pence and Elder (4) have noted that a purified albumin fraction prepared from wheat flour was electrophoretically heterogeneous. Ultracentrifuge studies had indicated the components were of similar molecular size. The electrophoretic patterns of rice albumins showed greater spreading than those of wheat. This may be a characteristic of rice proteins, or possibly the wheat proteins were more highly purified and small amounts of minor constituents removed.

In the water-soluble fraction, 36% of the nitrogen was nonprotein nitrogen as determined by trichloracetic acid precipitation of the protein. This corresponds to a nonprotein nitrogen value of 3.5% of the total nitrogen content of the brown rice. This value is slightly lower than those of Jodidi (1), who found the nonprotein nitrogen varied from 4 to 5% of the total nitrogen. Undoubtedly this value depends not only upon the rice variety and environmental conditions but also upon storage conditions. Proteolytic enzymes in the rice kernel and the effects of microbiological contaminants could alter the nonprotein nitrogen content under suitable conditions. The presence of the high amount of nonprotein nitrogen in the aqueous extract (about one-third of the total nitrogen therein) reflects upon the heterogeneity of this extract.

In conclusion, these investigations show that the water-soluble fraction of brown rice is highly heterogeneous. This is emphasized by the high nonprotein nitrogen content of the extract as well as by its electrophoretic behavior.

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THE APPLICATION OF ENZYMES IN PRE-FERMENT PROCESSES FOR BREAD PRODUCTION[1]

LEO P. CARROLL,[2] BYRON S. MILLER,[2] AND JOHN A. JOHNSON[2]

ABSTRACT

The activity of both malted wheat flour and fungal (*Aspergillus oryzae*) alpha-amylase and protease in the American Dry Milk Institute (ADMI) and Fleischmann pre-ferments remained essentially constant for 24 hours of fermentation. In the sugar pre-ferment the alpha-amylase activity of both malted wheat flour and the fungal preparation declined rapidly after 30 minutes and 2 hours, respectively. No alpha-amylase activity from the malted wheat flour or the fungal preparation remained after 2.5 hours. In the sugar pre-ferment, after 6 hours the protease activities of the malted wheat flour and the fungal preparation were 75 and 50%, respectively, of the original. During the following 18 hours of fermentation there was only a slight loss of protease activity. The loss of both protease and alpha-amylase activity in the sugar pre-ferment apparently was due to a decrease in pH.

No difference in bread quality could be observed whether the enzyme supplements were added to the ADMI and Fleischmann pre-ferments or later at the dough stage. The optimum protease supplementation for the two pre-ferments appeared to be three times that required with the sponge method. The pre-ferment methods tolerated large quantities of protease.

The adaptability of a pre-ferment to bulk handling methods and the economy and control that such a system may offer (2) for bread production has been of considerable recent interest. Other advantages of a pre-ferment process include the elimination of the setting of individual sponges, saving of floor space, reduction in processing time, and reduction in labor costs.[3] The American Dry Milk Institute (ADMI) pre-ferment has attracted much attention and reportedly produces a satisfactory loaf of bread provided proper control is maintained throughout the operation (9). Other pre-ferments (4) utilize a formulated salt mixture to stabilize the acidity of the pre-ferment during fermentation. A few bakeries have employed a sugar, yeast, and water pre-ferment.

It seems reasonable that some pre-ferment system eventually may be used widely in commercial baking. Although both alpha-amylase and protease are generally accepted as beneficial adjuncts in the sponge

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[2] Department of Flour and Feed Milling Industries, Kansas State College, Manhattan, Kansas.

[3] Choi, R. P. New developments in the baking industry. Presented at the 39th annual meeting, Denver, Colorado, May 1954.

baking process, no information is available concerning their stability or utility in the relatively new pre-ferment baking processes.

The purpose of this study was to determine the stability of alpha-amylase and protease in different pre-ferment formulations. The optimum relative protease supplementation for pre-ferment systems was compared with that used in the sponge method of breadmaking.

Materials and Methods

A commercial fungal enzyme concentrate, Rhozyme A-4,[4] and malted wheat flour[5] were used as sources of alpha-amylase and protease. Diastase-33[4] was used as a source of extra alpha-amylase in the baking experiments. The alpha-amylase activities of the three preparations were 5322, 54, and 5136 SKB units (10) per g., respectively, and the protease activities were 33,000, 44, and 650 hemoglobin units (7) per g., respectively.

Six hard red winter wheat flours were used. The protein content ranged from 10.4 to 13.2% and the ash ranged from 0.39 to 0.44%, expressed on a 14.0% moisture basis. All flours were unmalted. Three were of good baking quality and three of poor baking quality.

The ADMI, sugar, and Fleischmann pre-ferment formulas used in determining the stability of alpha-amylase and protease are shown in Table I. The enzymes were added in the form of water extracts. The ingredients of each pre-ferment were mixed in a Waring Blendor for 1 minute, poured into Erlenmeyer flasks, and allowed to ferment at 30°C. (86°F.). They were shaken gently and continuously on a mechanical shaker.

The pH of each pre-ferment was measured with a Beckman Model H-2 pH meter each time, prior to determining the enzyme activity.

Alpha-amylase was determined by the modified Wohlgemuth procedure of Sandstedt, Kneen, and Blish (10). Aliquots were withdrawn from the pre-ferments at intervals and centrifuged at 2000 r.p.m. for 5 minutes. Aliquots of the supernatant liquid were used to determine alpha-amylase activity. The loss of activity was calculated on the basis of the activity determined immediately after mixing the pre-ferments.

Protease was determined by the modified Ayre-Anderson technique (5) with Bacto hemoglobin as a substrate. Ten milliliters of trichloroacetic acid solution containing 1.125 g. per ml. were used. The gelatin viscosity technique of Koch and Ferrari (3) also was employed. Aliquots were withdrawn from the pre-ferments at 3-hour intervals, cen-

[4] Rohm and Haas Co., Philadelphia, Pennsylvania.
[5] Kansas Milling Co., Wichita, Kansas.

TABLE I

PRE-FERMENT FORMULAS

INGREDIENTS	QUANTITY OF INGREDIENTS		
	ADMI (1)	SUGAR (3)	FLEISCHMANN (5)
	g.	g.	g.
Water	320	320	320
Dextrose	21	21	21
Yeast food[a]	3.5	...	...
Enzyme preparation[b]	...	...	...
Diammonium hydrogen phosphate	...	0.3	...
Brew improver[c]	...	...	2.1
Salt	7	7	7
Dry milk solids	42	...	...
Compressed yeast[d]	14	14	14

[a] Arkady type.
[b] Malted wheat flour and Rhozyme A-4.
[c] Standard Brands, Inc., New York 22, N. Y.
[d] Fleischmann's.

TABLE II

STANDARD SPONGE AND PRE-FERMENT BAKING FORMULAS

INGREDIENTS	QUANTITY OF INGREDIENTS		
	Sponge	Fleischmann (5)	ADMI[a] (1)
	g.	g.	g.
		Pre-Ferments	
Water	314	320	320
Dextrose	...	14	14
Dry milk solids	...	...	42
Yeast food[b]	3.5	...	3.5
Rhozyme A-4	[c]	[c]	[c]
Rhozyme-33	0.015	0.015	0.015
Brew improver[d]	...	1.4	...
Salt	...	9	14
Compressed yeast[e]	14	11	14
Flour	490	...	...
		Dough	
Flour	210	700	700
Dextrose	42	42	42
Shortening	21	21	21
Salt	14	5	[illegible]
Dry milk solids	42	42	...
Water	155	149	149
Rhozyme A-4	...	[c]	[c]
Compressed yeast[e]	...	14	7
Yeast Food[b]	...	3.5	...

[a] American Dry Milk Institute.
[b] Arkady type.
[c] See Table III.
[d] Standard Brands, Inc., New York 22, N. Y.
[e] Fleischmann's.

trifuged, and used to determine protease activity. The loss of activity was calculated on the basis of an original activity of 100%.

Both sponge-dough (1) and pre-ferment-dough baking procedures were employed. Rhozyme A-4 was used as the source of protease. Excess alpha-amylase (11 SKB units per 100 g. flour) provided by Rhoyzme-33 was used in all cases. The ingredients of the pre-ferments were mixed in a Waring Blendor for 1 minute, poured into Erlenmeyer flasks, and allowed to ferment 4 hours at 30°C. (86°F.). Fleischmann and ADMI pre-ferments were used. After 4 hours of fermentation both the sponges and pre-ferments were mixed into a dough. The doughs were handled similarly except for a longer mixing time given the pre-ferment doughs. The pre-ferment doughs also were given 10 minutes and 5 minutes additional intermediate and final proofs, respectively. Both the sponge and pre-ferment doughs were proofed to a constant height before being placed into the oven. The formulas for the sponge and pre-ferments are shown in Table II.

Results and Discussion

The changes in the alpha-amylase activity with the different pre-ferments are shown in Fig. 1. The activity of Rhozyme A-4 and malted

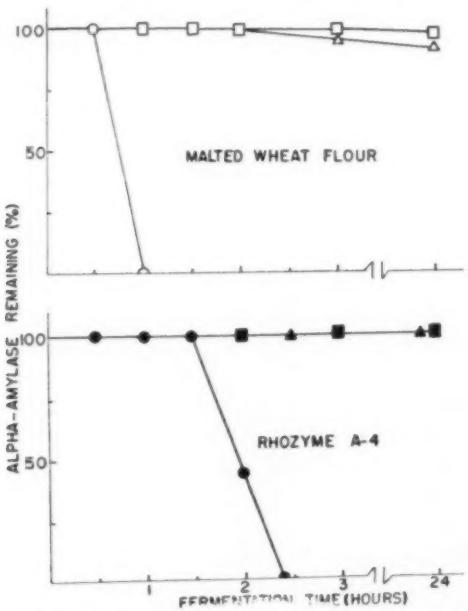

Fig. 1. Effect of fermentation time on the amount of alpha-amylase activity retained in different pre-ferments. Open and closed symbols represent the use of malted wheat flour and Rhozyme A-4, respectively. The triangles, circles, and squares represent ADMI, sugar, and Fleischmann pre-ferments, respectively.

wheat flour remained essentially constant in the ADMI and Fleischmann pre-ferments. The alpha-amylase activity declined rapidly in the sugar pre-ferment, however. This apparently was due to the development of high acidity, since no buffer was present in the sugar pre-ferment. The changes in pH with time for the different pre-ferments are shown in Fig. 2. They are similar to those reported by Johnson and

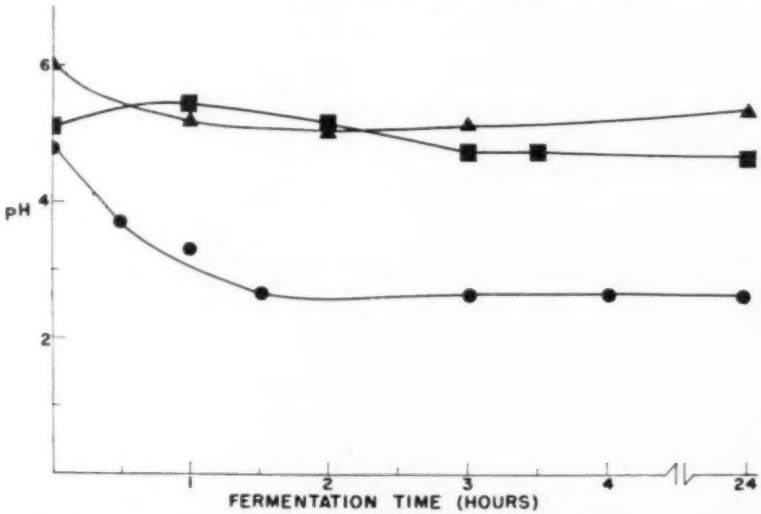

Fig. 2. Effect of fermentation time on pH of pre-ferments. The triangles, circles, and squares represent ADMI, sugar, and Fleischmann pre-ferments, respectively.

co-workers (2). The milk served as a buffer in the ADMI pre-ferment, and salts present in the brew improver provided the buffering effect in the Fleischmann pre-ferment. The changes in pH with time for the ADMI and Fleischmann pre-ferments were similar to those reported previously for the ADMI process (see footnote 3). The pH of the Fleischmann pre-ferment, however, tended to be 0.4 to 1.0 lower in pH than that for the ADMI pre-ferment.

The losses of protease activity in the different pre-ferments are shown in Fig. 3. The decreases in activities of Rhozyme A-4 and malted wheat flour protease in the Fleischmann and ADMI pre-ferments were slight over a 24-hour period. The protease activity in the sugar pre-ferment declined rapidly to 50 and 75% of the original for Rhozyme A-4 and malted wheat flour, respectively, during the first 6 hours and then remained nearly constant for the remaining 18 hours of the fermentation period. A very similar trend was observed when protease activity values determined by the gelatin viscosity technique were

plotted. The work of Miller and Johnson (6) suggests that the decrease in protease was due to the high acidity developed in the sugar pre-ferments (Fig. 2) in which no buffer was present.

The stabilities of alpha-amylase and protease were studied in pre-

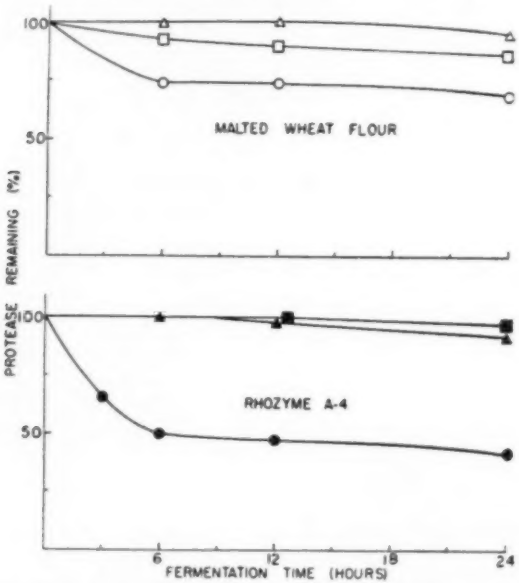

Fig. 3. Effect of fermentation time on the amount of protease activity retained in different pre-ferments. Open and closed symbols represent the use of malted wheat flour and Rhozyme A-4, respectively. The triangles, circles, and squares represent ADMI, sugar, and Fleischmann pre-ferments, respectively.

ferments maintained at 30°C. (86°F.). Although the ADMI process specifies pre-fermentation at 38°C. (100°F.), it is believed that similar stability results would have been obtained at the higher temperature. Miller, Johnson, and Palmer (8) found that the fungal and malted wheat flour amylases were stable at least to 65°C. (149°F.). Miller and Johnson (6) found that the fungal and malted wheat flour proteases at pH 5.0 were stable during 30 minutes of heating at 50°C. (122°F.). Furthermore, the analyses of protease activity are usually conducted at 40°C. (104°F.).

The enzyme activity decreased markedly in the sugar pre-ferment. Preliminary experiments showed that inferior bread resulted when this pre-ferment was employed. Accordingly, further experiments using the sugar pre-ferment were not performed.

The data in Table III summarize the results for the sponge and ADMI processes using various concentrations of Rhozyme A-4 with

strong and weak flours. While results obtained with the Fleischmann process are not presented, the trends were similar to those for the ADMI process. The sponge method, as might be expected because of

TABLE III

SUMMARY OF BREAD SCORES FOR BREAD BAKED USING ADMI PRE-FERMENTS AND SPONGE METHODS WITH THREE WEAK AND THREE STRONG HARD RED WINTER WHEAT FLOURS

RHOZYME A-4	STAGE ENZYME ADDED	BREAD SCORES[a]	
		Flours of Good Baking Quality	Flours of Poor Baking Quality
mg./700 g. flour			
		Sponge method	
0	Sponge	90.0	86.7
6	Sponge	95.0	...
9	Sponge	94.0	87.7
12	Sponge	93.0	...
27	Sponge	...	78.0
		ADMI[b] pre-ferment process	
0	Pre-ferment	92.5	74.0
9	Pre-ferment	93.8	74.7
18	Pre-ferment	94.5	...
27	Pre-ferment	94.3	78.7
9	Dough	92.0	76.7
18	Dough	93.3	...
27	Dough	93.3	78.0

[a] Volume, 20; break and shred, 5; crumb color, 5; grain and texture, 35; absorption, 10; dough handling properties, 15; mixing tolerance, 10; total, 100 points.

[b] American Dry Milk Institute.

the longer time for enzyme action, was much more sensitive to the action of protease. Using the sponge method, the flours tested required 6 to 9 mg. of Rhozyme A-4 per 700 g. of flour to produce the best-quality bread. In the absence of added enzyme supplement, the bread lacked volume, satisfactory break and shred, and grain and texture. Excess enzyme supplementation was even more damaging to these bread attributes.

With the pre-ferments, whether the enzyme was added to the pre-ferment or at the dough stage, the Rhozyme A-4 requirements for the flours were not critical. Satisfactory bread was produced using as high as 27 mg. of Rhozyme A-4 per 700 g. of flour. On the average, three times as much enzyme is required for the pre-ferment processes as for the sponge process. The use of Rhozyme A-4 was beneficial in all cases.

Acknowledgment

The technical assistance of Mr. Donald Miller, Kansas State College, and the financial assistance by Rohm and Haas Company, Philadelphia, are gratefully acknowledged.

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A METHOD OF ANALYZING EXTENSOGRAMS OF DOUGH[1]

Ben Grogg[2] and Diane Melms[2]

ABSTRACT

A method based on the evaluation of the slope and intercept of the line of minimum slope has been applied to the analysis of extensograms at constant extension. This method gives information on the elastic and viscous properties of dough. Variations in the absorption and salt content of dough have been used to demonstrate the application of the method.

As the absorption or salt level was increased, the viscous property of the flour was decreased. The elastic property of the flour was increased by salt. The influence of absorption upon the elastic property was dependent upon the salt level used.

Several dimensions of the extensogram have been used to characterize the physical properties of wheat flour doughs (1, 5, 6, 7, 8). The most recently advocated dimension is the tension at constant extension (5). It is conceivable that two or more doughs may give the same ten-

[1] Manuscript received August 5, 1955.

[2] The Quaker Oats Company Research Laboratories, Barrington, Illinois.

sion at constant extension, but have differently shaped extension curves or "pathways" by which the tension was developed.

A preliminary study herein reported indicates certain advantages to analyzing the extension curve for its shape. In ideal rheological systems, for example textiles (2), the slope and intercept of the line of minimum slope of the extension curve are used as measures of the elastic and viscous elements, respectively. Although wheat flour doughs are nonideal (3), it is possible to gain insight of the rheological properties by applying the techniques developed for ideal systems.

The extensogram is the plot of resistance to extension or tension versus extension. While this is not a stress-strain curve, it is possible to convert it to one by correcting for the decrease in cross-sectional area as the dough cylinder is extended.

A brief study of absorption and salt variations in doughs has been made to demonstrate a means of analyzing the resulting extensograms in a manner which takes into account the shape of the curve.

Materials and Methods

An unbleached straight-grade flour milled from hard winter wheat was used for this study. The protein content was 11.7% and the farinograph absorption (500 units) was 63.2%. Doughs were prepared using 0, 1, 2, 3, and 4% salt (based on weight of flour) at absorptions of 58, 60, 62, and 64%. The dough was mixed for 1 minute in a National pup dough mixer using 200 g. (14% moisture basis). The salt was dissolved in the water used. Aliquots of 150 g. each were scaled from the mixed dough, rounded 20 turns, molded, and saddled. The saddled doughs were arbitrarily given a 30-minute rest period in the extensograph cabinet prior to extension. All materials and the extensograph were maintained at 25°C. throughout the study. The dough cylinders were extended 5 cm. as indicated by the kymograph paper.

Results and Discussion

In Fig. 1 some of the resulting curves are shown. No correction was made for the unequal extensions of doughs offering different tensions. While the error introduced by not correcting is appreciable, it is also systematic (4).

It was assumed that the average cross-sectional area of the dough cylinders before extension and the change in cross-sectional areas during extension were both approximately the same for all samples. This assumption is the subject of current studies and is of importance since the cross-sectional area is needed to convert the extensogram to a stress-strain diagram.

The effect of variations in salt and absorption on dough properties are well established and understood by those in the trade. It is implied by the descriptive terms used in the literature that salt and absorption

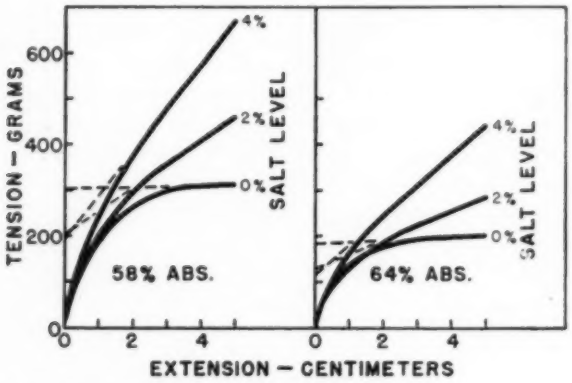

Fig. 1. Sample extensograms showing the method of analyzing the line of minimum slope.

may be associated with two different systems in the dough. It is possible to verify this implication quantitatively by analysis of the curve shape.

The extensograms were analyzed by determining: 1) the tension developed at 5 cm. extension, 2) the slope of the line of minimum slope, and 3) the intercept of the line of minimum slope at zero extension.

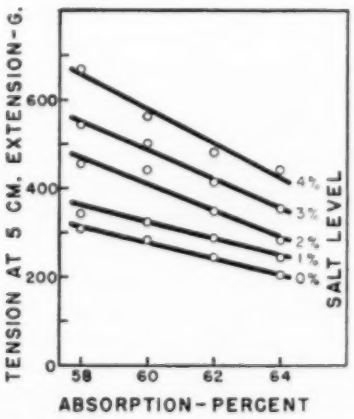

Fig. 2. The effect of absorption and salt on the tension at 5 cm. extension.

In Fig. 2 the tension at 5 cm. extension is plotted against the absorption used for each salt level. When the absorption was increased the tension decreased. At any given absorption, the addition of salt brought about an increased tension. Inspection of Fig. 2 shows that it is possible to develop the same tension at a constant extension in two or more doughs by adjusting the absorption and salt level. For example, to develop a tension of 300 g. at 5 cm. extension, an absorption of about 58.8% with no salt, 61.3% absorption with 1% salt, or about 63% absorption with 2% salt could be used. It is possible, however, to differentiate these doughs by analysis of the slope and the intercept of the line of minimum slope.

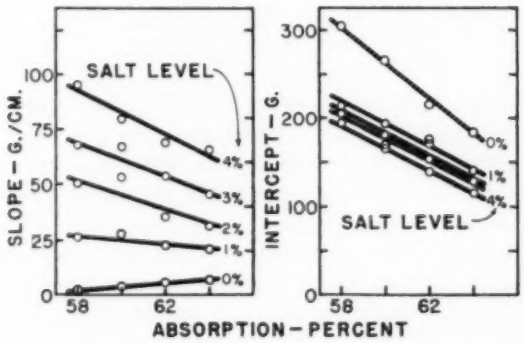

Fig. 3. The effect of absorption and salt on the slope and intercept of the line of minimum slope.

In Fig. 3 the slope of the line of minimum slope and the intercept of the line of minimum slope at zero extension are plotted against the absorption used with each salt level. With no salt in the dough the slope value increased very slightly when the absorption was increased, all slope values being quite low. With 4% salt in the dough the slope value decreased when the absorption was increased, while all the slope values were high as compared to those obtained with no salt. The intermediate salt levels gave intermediate relationships. At a given absorption, increasing the salt level appeared to increase the slope value in an approximately linear manner, the effect being more pronounced at the lower absorption and becoming less pronounced at the higher absorption. This becomes more apparent if the slope values are plotted as a function of the salt concentration for each of the absorptions used.

The intercept of the line of minimum slope decreased when the absorption and/or salt level was increased. The relationship between

absorption and intercept appeared to be approximately linear, whereas the relationship between salt and intercept appeared more complex since the addition of 1% salt brought about a pronounced drop in the intercept; further additions of salt had less effect.

The slope and intercept of the line of minimum slope for the three examples of dough conditions which gave 300 g. tension at 5 cm. extension are listed below:

Absorption %	*Salt Level* %	*Slope* g/cm	*Intercept* g.
58.8	0	2.5	285
61.3	1	23.5	178
63.7	2	33.5	136

The slope value increased about 13 times in changing from an absorption of 58.8% with no salt to 63.7% with 2% salt, while the intercept decreased about 48%. It would seem that the slope is associated with the elastic property of the dough, whereas the intercept is associated with the viscous property.

While this method of analysis leaves much to be desired, since the cross-sectional area of the dough cylinder was not compensated for, it would appear that it has value in the interpretation of extensograms. By analyzing in this manner it is possible partially to separate the composite effects which contribute to the shape of the extensogram and gain fundamental information using a commercially available instrument.

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THE USE OF A STARCH-IODINE-BLUE TEST AS A QUALITY INDICATOR OF WHITE MILLED RICE[1]

JOHN V. HALICK[2] AND KENNETH K. KENEASTER[3]

ABSTRACT

A rapid and simple method involving use of a starch-iodine-blue test to measure varietal differences in white milled rice is described. The intensity of the blue color produced is thought to be indicative of the cooking and processing quality of the rice. A low reading was given by varieties known to be of superior quality, while varieties known to be of poor quality gave high readings. A good correlation was found between the results of this method and actual cooking tests. Among the advantages of the test are its relative ease and rapidity, and the fact that only a small sample is needed. The iodine test is thought to be more sensitive than other less objective methods and permits sharper distinctions between rice varieties having good, intermediate, and poor cooking and processing characteristics.

Consumer preference in the United States is for long-grain rice, which is dry and fluffy with a minimum of stickiness and splitting when properly cooked, although there is some demand for the short and medium-grain types. However, long-grain rice varieties now being grown in this country vary greatly as to cooking behavior. In addition, food processors in recent years have experienced difficulties in obtaining regular supplies of rice having the specific properties required by their processes. A simple and rapid test for culinary quality, utilizing a small amount of grain, is needed by rice breeders and processors of milled rice. Such a test is particularly necessary in a breeding program to permit early elimination of varieties and selections of inferior quality.

Warth and Darabsett (4) and later Jones (1) observed varietal differences in rice when the grains were immersed in a dilute solution of potassium hydroxide and proposed the "alkali test" as a quality indicator of rice. It was reported that varieties in which the kernels disintegrated into clear and intermediate masses were of better cooking quality than those that disintegrated into opaque masses. More recently, Rao *et al.* (2) have employed "swelling numbers" as an index of cooking quality in rice. The swelling number was described by these workers as the weight of the water imbibed by 100 g. of rice when cooked in water at 98°C. under standard conditions.

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[3] Technologist, Converted Rice, Inc., Houston, Texas.

In the present paper a method is described involving use of a starch-iodine-blue test by which it is possible to detect varietal differences in milled rice. Differences in rice varietal behavior based on water-uptake are also described.

Materials and Methods

Starch-Iodine-Blue Test. A 1-g. sample of milled rice (ground to pass through a 0.5-mm. screen) is transferred to a 250-ml. Erlenmeyer flask. Exactly 100 ml. of distilled water are added and the flask and contents immersed for 45 minutes in a water bath maintained at 77°C. The sample is then removed from the bath, allowed to stand at room temperature for 15 minutes, and filtered through Whatman No. 12 filter paper. The first 30–40-ml. portion of the filtrate is discarded. A 10-ml. aliquot of the filtrate is pipetted into a 100-ml. volumetric flask containing 1 ml. of iodine solution (2 g. iodine and 20 g. potassium iodide in 1 liter of solution), 1 ml. of 30% hydrochloric acid solution, and approximately 60–70 ml. of distilled water. The flask is diluted to the mark, shaken, and allowed to stand at room temperature for at least 30 minutes. The intensity of the blue color is determined in a photoelectric colorimeter at 600 mu. Percent transmission is set at 100 with a solution of 1 ml. of iodide and 1 ml. acid solution in 100 ml. distilled water.

Water-Uptake Studies. In the water-uptake studies a metal tea bag containing 8 ($\pm$ 0.01) g. of polished rice is placed in 500 ml. of boiling distilled water for 15 minutes. The sample is then immersed in iced distilled water for 3 minutes, drained, and weighed. The results are expressed as the number of grams of water imbibed by 100 g. of rice.

Results and Discussion

The starch-iodine-blue test was modified from a method used by Roberts *et al.* (3) to estimate the degree of parboiling in rice. One of the authors (K. K. K.) observed that the test was also useful in detecting varietal differences in parboiled (converted) rice. It was later found that by "cooking" the sample for 45 minutes at 77° C. the value of this test was extended for use with milled raw rice. The reaction mixture is acidified to stabilize the color-complex.

Typical results obtained with the iodine test along with swelling numbers are shown in Table I. These results can be considered comparable, since all varieties were grown under similar conditions. The lowest readings were given by Texas Patna and Rexoro, varieties known to be of superior cooking and processing quality. The highest reading was obtained with Century Patna 231, which is inferior in

cooking quality and is unacceptable by manufacturers and users of processed rice. The sample of Bluebonnet 50 produced a reading somewhat higher than that of Texas Patna and Rexoro. This variety is

TABLE I
IODINE-BLUE VALUES AND SWELLING NUMBERS OF RICE SAMPLES

VARIETY	TRANSMISSION	SWELLING NUMBER
	%	
Long-grain		
Texas Patna	17.5	207
Rexoro	19.0	203
Bluebonnet 50	28.5	208
Century Patna 231	78.0	209
Short-grain		
Caloro	42.5	184
Medium-grain		
Blue Rose	50.0	174
Zenith	40.5	...

limited in its use by processors, but is known to have acceptable cooking quality. The short-grain variety, Caloro, and the medium-grain varieties, Blue Rose and Zenith, produced intermediate readings. On 57 replicate determinations, the standard deviation was found to be 1.3 and the standard error 0.171. The starch "blue values" of Roberts *et al.* (3) were reported to be indicative of the amount of soluble amylose in parboiled rice. The results reported herein lend some support to the general opinion that a high amylose content of rice is associated with superior cooking and processing characteristics.

It is emphasized that the differences between the varieties are relative and the actual readings vary with the conditions of the test. The most critical factor in the procedure outlined above is the temperature of the water bath. Under the conditions of this laboratory the water temperature was maintained between 77.0° and 77.4° C. A deviation of only 1.5° C. from this temperature was sharply reflected in the readings of the different varieties, although the relative differences between them remained approximately the same. Cooking time, porosity of the filter paper, and particle size of the sample also influence the transmission values.

Swelling number determinations exhibited no differences between the long-grain varieties. However, differences were observed between the short-medium and long-grain types. The swelling numbers could be determined with an accuracy within 5%. The greatest source of

error is thought to be the removal of excess water before the final weight is taken. Rao *et al.* (2) reported an accuracy of 1%; however, these authors used moist chamois leather to remove surplus water from the cooked rice. Since there were no differences in swelling numbers of rice varieties known to vary greatly in cooking and processing characteristics, no attempt was made to improve the accuracy of this method.

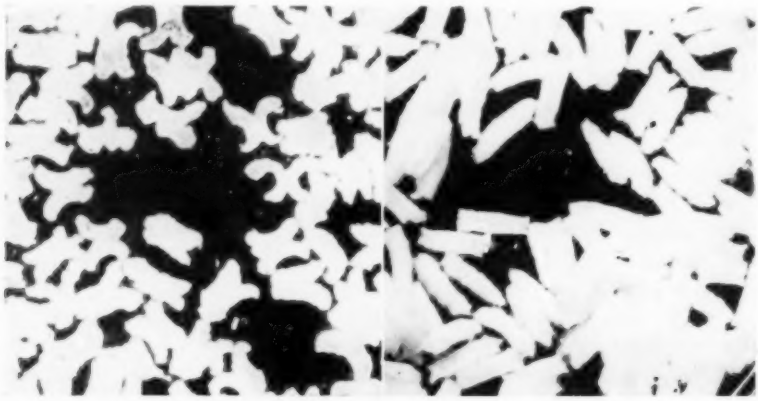

Fig. 1. Century Patna 231, left, a variety known to have poor cooking and processing characteristics, shows extensive splitting after cooking and soaking. Rexoro, right, a variety known to have acceptable cooking and processing characteristics, shows a uniform appearance after cooking and soaking.

In conjunction with the iodine test, a simple cooking test was also performed. Twenty-three milliliters of distilled water were added to a 250-ml. beaker containing 10 ml. of milled raw rice. The sample was cooked in an autoclave in a steam atmosphere without pressure for exactly 20 minutes. At the end of this time all of the water had been absorbed by the rice in most of the samples. A portion of the cooked rice was placed in a Petri dish, submerged in distilled water, and soaked overnight. After soaking, the rice samples were critically examined and photographed. Figure 1 illustrates the extremes observed between varieties having good and poor cooking characteristics. Varieties known to be of poor quality (left) consistently have every grain split longitudinally with the ends curled. On the other hand, varieties with good quality (right) present more uniform appearance and the grains remain intact. It should be pointed out that varieties that split after soaking also exhibit the greatest degree of stickiness when cooked according to conventional methods. The results of this cooking test on 200 individual samples of hybrid material were in agreement with the

iodine test in 86% of the samples; the results in 8% were indeterminate, and there was no agreement in 6% of the samples.

Among the advantages of the iodine test are its relative ease and rapidity, and the fact that only a small sample is needed. The latter fact is especially important in a breeding program, since there is not enough grain for adequate cooking tests in the early generations. Although the swelling number determination has been used by the Indian workers (2) as a quality indicator of rice, preliminary results indicate that this method is not reliable for American varieties. Furthermore, the iodine test is thought to be more sensitive than the "alkali test" which is less objective. The iodine test permits sharper distinctions between rice varieties having good, intermediate, and poor cooking and processing characteristics.

Acknowledgments

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DETERMINATION OF THE DEGREE OF POLISHING IN RICE

IV. Percentage Loss of Phosphorus as an Index of the Degree of Milling[1]

H. S. R. DESIKACHAR

ABSTRACT

Phosphorus and thiamine determinations in samples of rice polished to different degrees showed that the general pattern of loss was the same for both the nutrients. For a thiamine loss of 50%, the corresponding loss of phosphorus was about 55%. It is thought, therefore, that the percentage loss of phosphorus during the milling may be used as a check to control the degree of polishing of rice in rice mills.

The object of undermilling rice is mainly to conserve its thiamine content. The development of a simple and quick colorimetric method for thiamine assay is therefore very desirable for processing control in rice milling. As such a method for routine use in rice mills is not available at present, alternative indirect procedures that measure the loss of other nutrients or constituents in rice during milling are also useful and have been suggested from time to time (1, 2,4, 6, 7). The determination of phosphorus in rice for control of milling is such an indirect method. Possible objections against the use of this method are that phosphorus is not a limiting nutrient in typical rice diets and that the phosphorus content of rice is not a good index of its thiamine content. Because of varietal difference in thiamine and phosphorus content and the extent of their loss during milling, it was not possible in earlier work (1, 2) to demonstrate clearly the relations between the loss of thiamine and phosphorus during milling. However, if the phosphorus content is measured as a percentage of that present in the original unpolished rice and if the percentage loss during milling is correlated with the corresponding percentage loss of thiamine, then the phosphorus content of the rice can be taken as an index of its degree of milling. The present investigation was undertaken with the above objects in view.

The high thiamine and phosphorus content of rice bran and the data on the loss of nutrients during washing of rice (11) indicate a concentration of these constituents in the peripheral layers. For this reason, the pattern of their loss during milling is expected to be similar. To emphasize this similarity about their unequal distribution in

[1] Manuscript received January 23, 1956. Communication from the Division of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, India.

the grain, data on the distribution of phosphorus in the bran, germ, and endosperm of the rice grain were collected, using the dissection technique adopted by Hinton (8) for his studies on thiamine distribution. Values for different forms of phosphorus in rice bran and in polished rice have been reported recently by McCall *et al.* (10).

Materials and Methods

The six varieties of paddy chosen for milling were S-749, S-661, H-497, S-199, China 45, and GEB 24. They were obtained from a paddy breeding station and the shelled rice from the six varieties was found to contain in respective order 3.6, 3.9, 3.1, 4.2, 3.6, and 4.1 γ per g. of thiamine and 238.7, 259.6, 286.2, 296.5, 290.2, and 213.7 mg. per 100 g. of phosphorus. The first three varieties of paddy were first shelled and samples of rice polished to different degrees were obtained from the shelled rice by passing it through a commercial polishing cone. Samples of polished rice were obtained from the last three varieties using a "huller" type of equipment in which the shelling and polishing were done in one operation. Fine control of the degree of polishing was not, however, possible with this equipment.

For studying the distribution of phosphorus, rice was stored for 3–4 days in a humid chamber to make it soft enough for dissection; about 100 grains in each of two varieties were dissected according to the method of Hinton (8); and the pooled germ, bran, and endosperm were used for phosphorus estimations. Thiamine and phosphorus were determined in the samples of rice milled to different degrees.

Thiamine was determined by the thiochrome method as modified by Kik and Williams (9) for extracting the thiamine. Phosphorus was determined by the colorimetric method of Fiske and Subbarow (3) using the wet digestion method of Gerritz (5) for complete oxidation of organic material.

Results and Discussion

About 50% of the phosphorus in the rice is present in the bran itself, while the rest is distributed between the germ and endosperm (see table below). The distribution of phosphorus obtained here differs in

Distribution of Phosphorus in the Rice Grain

	Red rice (Kar variety)	White rice (S. 139)
	%	%
Germ	7.9	9.7
Bran	47.9	51.1
Endosperm	44.2	39.2

some details from the pattern of thiamine distribution reported by Hinton (8), but in both cases it is important to note the high concentration of the nutrients in the bran layers.

TABLE I

RELATIVE LOSS OF THIAMINE AND PHOSPHORUS DURING PROGRESSIVE POLISHING OF RICE

SAMPLE NO.[a]	THIAMINE	PHOSPHORUS	THIAMINE	PHOSPHORUS	THIAMINE	PHOSPHORUS
	%	%	%	%	%	%
	A. Milled in a polishing cone					
	S-749		S-661		H-497	
1	8.3	11.8	10.6	12.3	20.3	19.2
2	24.7	30.5	28.7	29.8	30.0	29.7
3	32.1	37.2	45.6	49.7	45.6	48.1
4	50.4	52.8	59.4	62.5	...	...
5	54.1	55.6	65.6	67.1	...	...
6	62.4	64.3	...	...	...	...
	B. Milled in a huller-type machine					
	S-199		China 45		GEB-24	
1	43.4	50.0	41.2	43.4	29.0	32.1
2	55.4	61.7	56.4	60.7	50.5	54.5
3	68.4	73.1	60.8	62.2	56.4	60.1
4	...	...	...	...	59.8	68.4

[a] The extent of polishing increases with sample number.

The data on the loss of phosphorus and thiamine as a result of progressive milling (Table I) show that irrespective of the variety of paddy, the pattern of loss of the two nutrients when expressed as a percentage of that present in the unpolished rice follows a similar course. However, phosphorus tends to be removed slightly faster than thiamine during the course of polishing. This is especially true of samples polished in a "huller" type of equipment. At a stage of polishing when there is about 50% loss of thiamine, the loss of phosphorus is nearly of the same order (about 55%) in the different varieties studied.

To prevent the occurrence of beriberi in rice-eating countries, a level of polishing to retain 1.5–1.8 γ thiamine per g. in the rice is considered adequate and has been accepted as a safe degree of polishing (2). This would correspond to retention of about 50% of the thiamine or about 45% of the phosphorus in the polished rice. There is a varietal difference in both thiamine and phosphorus and hence it is desirable to express the degree of polishing in terms of the percentage loss of the nutrients as a result of polishing, rather than in terms of their absolute content in the polished rice.

Phosphorus has been estimated in the present study by using the complete digestion procedure of Gerritz (5) for oxidizing the phosphorus. A complete assay of the phosphorus content of the polished and the original unpolished rice by this method can be determined within 30 minutes. It has been shown earlier (2) that a short 5-minute digestion with sulfuric acid would release 90–95% of the phosphorus in the rice. If this short digestion procedure be adopted, it would be possible to complete an assay within about 20 minutes. The method therefore holds promise of application for use in rice mills for routine control of the degree of polishing.

Acknowledgments

The author is deeply indebted to Dr. V. Subrahmanyan, Director of the Institute, and to Dr. M. Swaminathan, Head of the Division of Biochemistry and Nutrition, for their keen interest in the work and their kind encouragement during the course of the investigation.

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CHANGES LEADING TO IMPROVED CULINARY PROPERTIES OF RICE ON STORAGE[1]

H. S. R. DESIKACHAR

ABSTRACT

Fresh rice, upon cooking, lost more solids into the cooking water than stored rice; it cooked to a pasty consistency, yielding a viscous gruel.

Solutions of amylose and starch isolated from fresh rice had a slightly higher specific viscosity than the corresponding constituents from old rice. The amylose from the new rice also exhibited a lower iodine-combining capacity when titrated potentiometrically with iodine. The water-imbibing capacity of fresh rice was greater than that of stored rice.

Habitual rice eaters are aware of the poor cooking quality of rice immediately after harvest. Such rice cooks to a pasty consistency, does not swell to the maximum extent while cooking, and is also alleged to cause digestive disorders (7). The cooking quality and digestibility improve on storage (7). The lowered amylase content and colloidal changes brought about during storage are considered to be responsible for the better cooking quality of the stored grain (4, 7). The present investigation relates to a more detailed study of the problem and was undertaken to explain the poor cooking behavior of fresh rice.

Initial experiments showed that steaming of fresh rice for 30 minutes destroyed about 95% of its amylase activity but did not prevent its pastiness on cooking. Soaking of the rice in an active amylase solution prepared from germinated ragi (*Eleucine coracana*) for 30 minutes and slowly cooking the rice in the same solution did not induce pastiness in the rice. In fact, the viscosity of the gruel was reduced by such treatment. This indicated that factors besides the high amylase content of fresh rice may be responsible for its pastiness on cooking. There was no difference in the acidity or pH of extracts of the old and the new rice. Addition of fatty acids to the cooking water reduced considerably the pastiness in cooked new rice, but when fatty acids combined with starch were actually estimated according to the method of Nussenbaum and Hassid (3) there was no difference between old and new rice. Experiments were therefore conducted to see whether the difference between fresh and old rice was due to any physico-chemical changes in the nature of starch, or changes in the affinity of the rice for water.

[1] Manuscript received January 23, 1956. Communication from the Division of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, India.

Materials and Methods

Five varieties of paddy were obtained immediately after harvest from a near-by paddy breeding station and were compared with respective control samples of old stored paddy of the same varieties from a previous harvest. The samples were highly milled (polished to remove 15–16% of the weight of the grain) and whole grains of rice were used for the cooking trials. Rice powdered to pass through a 60-mesh sieve was used for other studies. Samples freed of fat by acetone extraction were used for isolation of amylose.

Loss of solids in gruel during cooking was measured by steaming 5 g. of rice grains with 50 ml. water in a cooker for 30 minutes, straining the gruel through wire gauze, and evaporating the gruel to constant weight. For measuring the amount of solids leached by water, 5-g. samples of rice flour were extracted with warm water (50°–55°C.) repeatedly, the combined extracts made up to volume and filtered, and aliquots evaporated to dryness. The blue and the red color of the acidified extract with iodine were determined by a Klett-Summerson Photoelectric Colorimeter using filters Nos. 66 and 54 respectively.

The water-diffusible amylose component was prepared from China 45 variety by employing the method described by Meyer and Gibbons (2). After removal of the water-diffusible component, amylose was prepared from S-54, S-199, and China 45 varieties of rice by the butanol precipitation method of Schoch (6). The iodine-combining capacity of the amylose was measured by potentiometric titration according to Sanjiva Rao *et al.* (5). The total starch constituents of rice gruel were prepared by precipitation at 70% alcohol concentration after the gruel had been autoclaved and then centrifuged to remove insoluble material. The specific viscosity of isolated starch and amylose was determined in *N* potassium hydroxide solution as described by Lansky *et al.* (1), the viscosity of normal potassium hydroxide solution being taken as 1 for calculating specific viscosity.

Results and Discussion

Table I shows that under comparable conditions of cooking, new rice loses more solids into the cooking water than old rice, thereby yielding a thick viscous gruel. Warm water also extracts more solids from the fresh rice. The higher blue and red value of the aqueous extract of the new rice samples is evidence of higher amounts of diffusible starch and dextrins.

The specific viscosity in alkaline solution of the starch and amylose is greater for the fresh rice (Table II). The affinity towards water as

TABLE I

LOSS OF SOLIDS FROM RICE WHILE COOKING AND DURING EXTRACTION WITH HOT WATER

VARIETY	LOSS OF GRUEL SOLIDS	SOLIDS EXTRACTED BY HOT WATER	COLOR VALUE OF THE AQUEOUS EXTRACT WITH IODINE IN K.S. UNITS	
			Blue	Red
	g.	*mg.*		
S. 199: Old	0.60	60.3	108	117
New	0.95	78.6	197	138
S. 749: Old	0.68	109.3	64	84
New	1.00	135.4	102	112
S. 661: Old	0.64	65.3	68	71
New	1.12	72.7	115	106
S. 54: Old	0.74	59.0	88	103
New	1.09	70.2	147	145

TABLE II

SPECIFIC VISCOSITY OF AMYLOSE AND STARCH IN NORMAL POTASSIUM HYDROXIDE SOLUTION AT 35°C.

	AMYLOSE A_1,[a] 0.2% SOLUTION	AMYLOSE A_2,[b] 0.5% SOLUTION	TOTAL STARCH (FROM GRUEL), 0.2% SOLUTION
China–45			
Old	1.009	1.495	1.110
New	1.075	1.619	1.163

[a] Amylose A_1 refers to the water-diffusible starch.

[b] The amylose A_2 samples were tested for their 3.5 dinitrosalicylic acid reducing property for measuring their molecular chain length. No differences were noticed between old and new rice in this respect.

TABLE III

IMBIBITION OF MOISTURE BY OLD AND NEW RICE AT 27°C.

	NUMBER OF DAYS EXPOSED TO WATER VAPOR					
	1	2	3	6	8	10
	MOISTURE CONTENT					
	%	%	%	%	%	%
Old	11.7	14.0	15.2	17.6	19.8	22.0
New	11.3	14.3	16.0	18.0	19.9	22.2

TABLE IV

AFFINITY CHARACTERISTICS OF OLD AND NEW RICE WITH RESPECT TO WATER (CHINA-45)
(Average of six replicates is presented)

	WATER ABSORPTION		
	Paddy	Rice	Rice Flour
	%	%	%
Old	23.1	20.2	211
New	25.2	21.5	375

measured by the amount of moisture imbibed is also higher for the new rice (Table III). The same is also borne out by studies on the absorption of water when the rice, paddy, or rice flour is soaked in water (Table IV). Although the methods used for the water imbibition studies are empirical and are not highly reproducible, the data pertain to average values of six replicate experiments and can be taken as having significance.

The iodine-combining capacity of amylose from new rice is lower than that of old rice, as can be seen from the potentiometric titration curves presented in Fig. 1. The inflection of the titration curve or the

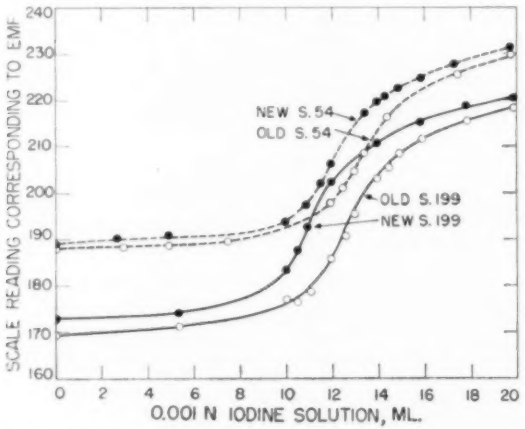

Fig. 1. Potentiometric titration with iodine solution of amylose from new and old rice.

point at which there is maximum change in electrical potential for a small addition of iodine solution occurs at an earlier stage in amylose from new rice. It is difficult to interpret the theoretical significance of the observed changes in the physico-chemical properties as a result of storage; the occurrence of such changes is, however, to be stressed. The observed changes during storage are in keeping with the colloidal theory of sol-gel transformation put forth by Sanjiva Rao (4).

The greater tendency to pastiness of new rice on cooking can be explained on the basis of the observations described above. There is a greater dispersal of starch granules in the cooking medium when new rice is cooked, and the higher specific viscosity of solutions of starch from the fresh rice would further contribute towards the pastiness. The causes for the greater dispersal of starch in the cooking water in the case of new rice need to be further investigated. It has been ob-

served that in the final stages of cooking, the new rice grain bursts along one of the lines of suture before dispersing its contents into the cooking water.

Paddy is usually harvested from the field at a stage when only 80–90% of the grains are fully mature (as judged by the hardness of the grain), for fear of shattering if allowed to mature more fully. Fresh rice therefore contains 10–20% of immature grains which may gradually set on aging and gain rigidity. The changes occurring during storage may well be a continuation of the ripening process. A study of changes occurring during ripening of the rice grains is needed to test this possibility.

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The author is deeply indebted to Dr. V. Subrahmanyan, Director of the Institute, and to Dr. M. Swaminathan, Head of the Division of Biochemistry and Nutrition, for their keen interest and kind encouragement during the course of this investigation.

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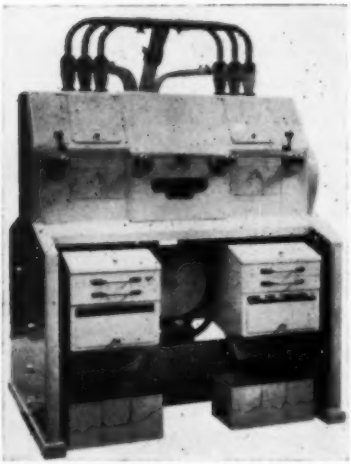

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